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THE ANTIOXIDANT AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF THE FLAVONOID-RICH FRACTION OF BAYAG-USA (*VOACANGA GLOBOSA)* LEAF EXTRACT

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ABSTRACT

Introduction: Dementia cases in the Philippines are projected to rise significantly, with Alzheimer's disease (AD) comprising 85.5% of cases (Anlacan et al., 2024). AD is linked to oxidative stress, characterized by an imbalance in reactive oxygen and nitrogen species and reduced acetylcholine activity. Voacanga globosa ("Bayag-usa") was evaluated for its antioxidant and acetylcholinesterase (AChE) inhibitory properties, which may help prevent neurodegeneration. **Methodology**: Leaf extracts were partitioned into methanol, hexane, ethyl acetate, and aqueous fractions. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, and saponins. Instrumental analysis and the Total Phenolics/Flavonoid Content Assay showed the ethyl acetate fraction was richest in flavonoids and phenolics. **Results**: Consequently, antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Cupric Reducing Antioxidant Capacity (CUPRAC), revealed weaker activity than ascorbic acid. AChE inhibition assay, though exhibiting a lower activity than galantamine, demonstrated a concentration-dependent effect, increasing from 15.12% at 100 ppm to 68.39% at 1000 ppm. Statistical analysis confirmed significant differences ($p < 0.05$) between V. globosa and the positive controls. **Discussion**: Despite its lower potency, the dose-dependent trend suggests potential bioactivity, warranting further research for therapeutic applications.

KEYWORDS*:* Voacanga globosa, flavonoids, acetylcholinesterase inhibition, antioxidant activity, in-vitro analysis.

CHAPTER 1

The Problem and its Background

INTRODUCTION

Neurological illnesses are closely linked to altered levels of acetylcholine (ACh) or abnormalities in receptor expression and function in specific regions of the nervous system, which disrupt normal neurotransmission and contribute to cognitive and motor deficits. These changes are often exacerbated by oxidative stress, which damages neuronal structures and impairs synaptic signaling. Additionally, oxidative stress can modulate acetylcholinesterase (AChE) activity, further disrupting cholinergic balance. This interplay highlights the central role of oxidative stress and AChE dysregulation in the progression of neurodegenerative diseases, underscoring their importance as therapeutic targets.

Neurological illnesses have been linked to a number of disorders, including Parkinson's, Alzheimer's, and Huntington's (Walczak-Nowicka & Herbet, 2021). For instance, severe cognitive deficits associated with Alzheimer's disease (AD) are caused by a loss of cholinergic tone in the basal forebrain cholinergic system, particularly projections to the cortex and hippocampus, as a result of degeneration of basal forebrain neurons (Bekdash, 2021). According to a study published by The Lancet Neurology, neurological conditions are currently the primary cause of illnesses and disabilities globally, with over 3 billion individuals living with neurological disorders in 2021. Since 1990, there has been an 18% increase in the total number of neurodegenerative diseases that result in impairment, illness, and premature mortality which is often referred to as disability-adjusted life years, or DALYs (Steinmetz et al., 2024). In the Philippines, the rise of dementia is expected to rise at 1,474,588 by 2030, 1,972,067 by 2040, and 2,529,436 by 2050, from 16 cases per 1,000 individuals in 2021 particularly AD which is accounted for 85.5% of all dementias, with vascular dementia coming in at 11.7% and other dementias at 2.7% (Anlacan et al., 2024).

Following decades of unsuccessful attempts to create effective strategies to prevent, prolong the onset, or decrease the development of the aforementioned disorders, acetylcholinesterase inhibitors were discovered to be useful in slowing the clinical progression of AD. Randomly assigned, placebo-controlled experiments in patients with mild to moderate AD and prodromal AD have demonstrated that AChE inhibitors have anti-neurodegenerative benefits in the basal forebrain, cortex, and hippocampus (Moss, 2020). Whilst the use of AChE inhibitors is well supported by research (e.g. donepezil, rivastigmine, galantamine), their effects on condition of living, cognitive abilities, global therapeutic states, and medical economic advantages have failed to achieve what is expected. This is particularly because of their inevitable gastrointestinal toxicity and short half-life, which restricts their usage to ineffectively low doses (Bekdash, 2021). As a result, continuous need for research and development of more effective therapies that minimize undesirable effects such as traditional medicine, is necessary to improve outcomes for patients.

Background of the Study

The Philippines is recognized as one of the most biologically diverse countries, with a rich array of medicinal plants valuable for both traditional use and the discovery of novel compounds for drug development. Despite its biodiversity and the long-standing use of medicinal plants, scientific research on endemic plants remains significantly underexplored (Meñiza et al., 2024).

Medicinal plants are well documented sources of bioactive compounds. Plant-derived natural products such as terpenoids, quinones, flavonoids, lignans, glycosides, coumarins, and alkaloids exhibit vast structural diversity, providing templates for developing new therapeutic strategies and drugs (Qin et al., 2022).

"Bayag-usa," scientifically identified as *Voacanga globosa* which is endemic to the country and is part of the Apocynaceae family. Traditionally, *V. globosa* is used to treat cancer, infections, and ulcers. In addition it also contains bioactive spirobisindole alkaloids such as globospiramine, deoxyvobtusine, and vobtusine lactone (Manzano et al., 2024). In anti-HIV studies, globospiramine and vobtusine lactone reduced HIV replication in cells by blocking the NF-kB activation pathway (De Jesus et al., 2022). Research has identified various bioactivities including anticancer, anti-mitotic, antimycobacterial, immunosuppressive, immunostimulatory, and anticholinesterase effects. While most studies focus on indole alkaloids, the potential of other phytoconstituents in *V. globosa*, such as flavonoids, remains underexplored (Qin et al., 2022).

Several plant-derived flavonoids have shown strong AChE inhibitory effects, studied extensively through in vivo, in vitro and in silico methods (Ismail Yener et al., 2020). However, the connection between the molecular structure of flavonoids and their inhibitory effects on AChE has not been thoroughly explored. Acetylcholinesterase (AChE) is a crucial enzyme in animals with cholinergic nervous systems. Acting as a serine hydrolase, AChE decomposes acetylcholine, a key neurotransmitter, into acetate and choline, helping to return the cholinergic neuron to its resting state (Li et al., 2020). Blocking this enzyme can increase acetylcholine levels in the brain, potentially improving cognitive function of patients with AD.

Oxidative stress is a prevalent problem in Alzheimer's disease (AD). Brain neurons are particularly susceptible due to their elevated oxygen use, which leads to amplified production of reactive oxygen species (ROS), combined with their relatively weak antioxidant defenses. Antioxidants from other parts of the body have difficulty crossing the blood-brain barrier (Li et al., 2022). In this regard, the capability of flavonoids to cross the blood-brain barrier implies that they may exert direct effects on the brain. Recognized for their strong antioxidant properties, flavonoids have the ability to neutralize the contributors to oxidative stress and neurodegenerative diseases such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). By minimizing oxidative damage, flavonoids contribute to the protection of neuronal health, which is essential in the context of AD. They also help modulate important physiological responses, which may enhance their neuroprotective effects in Alzheimer’s disease (AD). Unlike conventional drugs, flavonoids can be taken as dietary supplements, potentially providing early protection even at a young age due to their low toxicity. They can be safely used without a formal diagnosis, allowing for preventive use. However, further studies on long-term dietary intervention, including dosage and timing, are needed to evaluate their full potential for managing AD. Additionally, considering factors like bioavailability and metabolism throughout preclinical research could provide a clearer understanding of flavonoids’ effects within the body (Calderaro et al., 2022).

An effective approach for managing Alzheimer’s disease (AD) is to elevate acetylcholine levels in the brain by using acetylcholinesterase (AChE) inhibitors. Several AChE inhibitors have been researched for the management of AD, but only donepezil, tacrine, rivastigmine, and galantamine have been approved to use by the U.S. Food and Drug Administration (FDA). Although AChE inhibitors have been shown to enhance cognitive, functional, and behavioral symptoms of AD, they may also cause adverse side effects, such as gastrointestinal problems, cardiorespiratory issues,

extrapyramidal symptoms, genitourinary effects, musculoskeletal issues, and sleep disturbances (Zavala-Ocampo et al., 2022).

In this study, *Voacanga globosa* underwent sequential extraction with different solvents, and the flavonoid-rich extract was assessed for their in vitro antioxidant and acetylcholinesterase inhibitory activities.

Conceptual Framework

This research focused on the exploration of *Voacanga globosa* as a source of flavonoids with potential neuroprotective benefits. The key concepts of interest include its antioxidant activity and its ability to inhibit acetylcholinesterase enzymes which are highly relevant in addressing neurodegenerative disorders.

The researchers focused on determining the activity of the flavonoid-rich fraction of *Voacanga globosa* as these compounds are generally well-established for their antioxidant activities, protecting cells from oxidative damage. The choice to assess AChE inhibition of the plant is grounded in the understanding that inhibition of this enzyme to maintain acetylcholine levels is involved in improving synaptic function and neuroprotective benefits. This dual action of flavonoid-rich plants may offer an enhanced effect against neurodegenerative diseases.

The extraction of flavonoids and subsequent determination of their antioxidant and AChE inhibitory activities followed a logical framework based on phytochemical principles. Using maceration and solvent fractionation to isolate flavonoid-rich fraction reflected the alignment of the research study with phytochemistry principles aimed at extracting specific secondary metabolites. Chemical and physical tests for flavonoids identified its presence in the extracted fractions in a qualitative manner. FTIR as an instrumental analysis was also utilized to confirm the presence of characteristic functional groups of flavonoids in the different fractions. The Total Phenolic Content and Total Flavonoid Content determination helped quantify flavonoids and phenolics, which served as a basis for comparing fractions and identifying the flavonoid-rich fraction used in subsequent analyses. The DPPH Free radical scavenging assay, Cupric reducing antioxidant capacity, and AChE inhibition assay further validated the therapeutic potential of this flavonoid-rich fraction, allowing for both quantitative and qualitative assessments of in vitro activity.

Ultimately, this research aimed to determine the antioxidant properties and the AChE inhibitory activity of the flavonoid-rich fraction of *Voacanga globosa*.

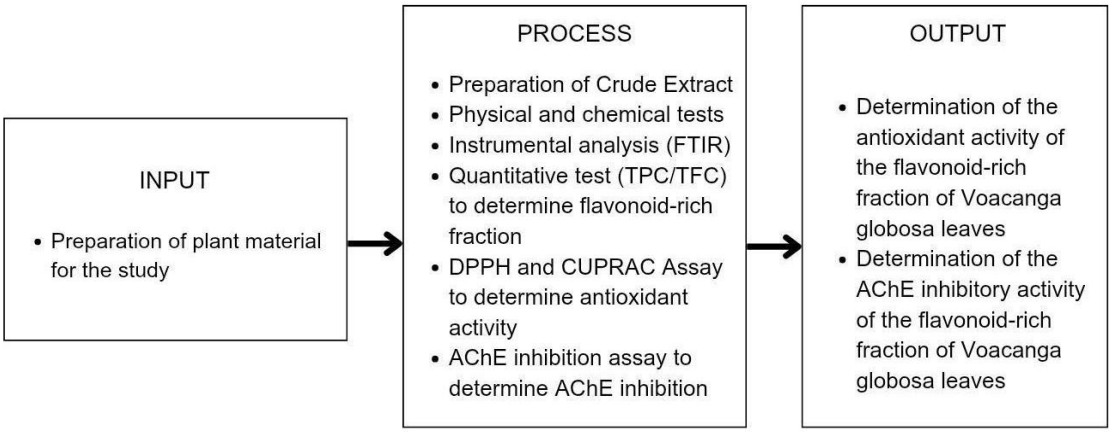

Figure 1: Operational Framework.

Research Objectives

This study sought to explore the potential antioxidant and Acetylcholinesterase inhibitory activity of the flavonoid-rich extract of the *Voacanga globosa* (Bayag-usa) leaves.

Specifically, it aimed to:

1. Determine the presence of secondary metabolites present in *Voacanga globosa* through phytochemical screening.

- Alkaloids
- Glycosides
- Saponins
- Flavonoids
- Tannins

2. Determine the percentage yield of the following extracts after solvent fractionation:

- Methanolic Extract
- N-hexane Fraction
- Ethyl acetate Fraction
- Aqueous Fraction

3. Determine which fraction has the highest phenol and flavonoid content through:

- Total Phenolic Content using Folin-Ciocalteu Method
- Total Flavonoid Content using Aluminum Chloride Colorimetric Assay

Determine the antioxidant property of the flavonoid-rich fraction of *Voacanga globosa* (Bayag-usa) using DPPH and CUPRAC Assay and compare its antioxidant property to the standard drug, Ascorbic Acid.

Determine the Acetylcholinesterase inhibitory activity of the flavonoid-rich fraction of *Voacanga globosa* (Bayag-usa) using AChE Inhibition Assay and compare to the Acetylcholinesterase Inhibition effects of the standard drug, Galantamine.

Hypothesis

Based on the research problems identified, the researchers formulated the following hypotheses:

Null Hypothesis (*Ho1*): There is no significant difference between the antioxidant property of the flavonoid-rich fraction of *Voacanga globosa* leaf extract and the standard drug Ascorbic Acid.

Alternative Hypothesis *(Ha1)*: There is a significant difference between the antioxidant property of the flavonoid-rich fraction of *Voacanga globosa* leaf extract and the standard drug Ascorbic Acid.

Null Hypothesis *(Ho2)*: There is no significant difference between the AChE Inhibitory Activity of flavonoid-rich fraction of *Voacanga globosa* leaf extract and the standard drug Galantamine.

Alternative Hypotheses *(Ha2)*: There is a significant difference between the AChE Inhibitory Activity of flavonoid-rich fraction of *Voacanga globosa* leaf extract and the standard drug Galantamine.

Significance of the Study

Neurodegenerative diseases affect millions of people worldwide with Alzheimer's and Parkinson's disease being the most common. This study is significant as it explores the potential therapeutic effects of Bayag-usa, a plant known for its traditional medicinal uses (National Institute of Environmental Health Sciences, 2022). This research delved into its flavonoid- rich extract, specifically assessing its antioxidant capacity and the inhibition of acetylcholinesterase (AChE) activity, which is crucial for conditions like Alzheimer's disease. Furthermore, the study's findings could benefit the following:

Drug manufacturers may use the collected data and the research findings in developing new drug products using Bayag- usa *(Voacanga globosa)*.

Patients with Neurodegenerative disorders can gain a positive attitude towards the use of Bayag-usa (*Voacanga globosa)* leaves as a safer natural alternative to high- cost medicines in the market.

Healthcare providers can further understand the antioxidant property and achE inhibitory activity of the flavonoid-rich fraction of the Bayag- usa (*Voacanga globosa)* flavonoid extract, and this herbal plant can be considered as a treatment for neurodegenerative disorders.

Future researchers may benefit from the result of the study as it may serve as their basis and reference for conducting related research.

Scope, Limitation, and Delimitation

This study aimed to investigate the potential acetylcholinesterase (AChE) inhibitory effects, along with antioxidant properties, of the flavonoid-rich fraction of *Voacanga globosa.* Investigations into the other potential pharmacologic effects of the flavonoids are omitted.

The experiment design focused specifically on the flavonoid-rich fraction derived from the leaves of *Voacanga globosa*, which was evaluated for its effect. This study exclusively examined the role of flavonoids; an analysis of the phytochemical activities of other constituents present in the plant, such as alkaloids, were not included. Furthermore, the research did not encompass the identification of the specific constituent that may be responsible for the observed acetylcholinesterase inhibitory and antioxidant effects. The study also refrained from the pure isolation of any particular constituent implicated in these bioactive effects.

Definition of Terms

To facilitate a comprehensive understanding of the study, it is essential to clarify and define the key terms and concepts used throughout. This section offers precise definitions and explanations, providing the necessary context for readers to accurately interpret the findings and analyses presented.

Acetylcholinesterase: It is a neurotransmitter or chemical messenger that is essential to brain and muscle function, which is hydrolyzed or broken down by a cholinergic enzyme to produce choline and acetic acid. It refers to the enzyme that is inhibited in the study (Trang and Khandhar, 2023).

Acetylcholinesterase Inhibition Assay: It refers to the colorimetric technique by utilizing Ellman's method that estimates the AChE activity. This assay is used to determine the acetylcholinesterase inhibition activity of *Voacanga globosa* (bayag-usa) leaves (Hanumanthappa et al., 2024).

Crude Methanolic Extract: It is a natural substance used to evaluate the bioactivity of phytochemicals. It refers to the extract collected from the leaves of *Voacanga globosa* by using 80% methyl alcohol.

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay: It refers to the spectrophotometric technique that provides basic information about the reactivity of compounds for the reduction of cupric ions (Cu2+) to cuprous ions (Cu+) by antioxidants present in the sample. This assay is used to determine the antioxidant property of *Voacanga globosa* (Bayag-usa) leaves (G-Bioscience, 2019).

DPPH Free Radical Scavenging Assay: It refers to the spectrophotometric technique that provides basic information about the reactivity of compounds for free scavenging radicals. This assay will be used to determine the antioxidant property of *Voacanga globosa* (Bayag-usa) leaves (Baliyan et. al., 2022).

Flavonoid-rich fraction: Fraction of the *Voacanga globosa* leaf extract obtained through solvent fractionation that contains the highest relative concentration of flavonoids compared to other fractions as determined by Total Phenolic/ Flavonoid Content Assay.

Oxidative Stress-Induced Neurodegenerative Disease: It is a collective illness that has an imbalance between pro-oxidant species and antioxidants, which is characterized by a decrease in endogenous antioxidant levels and an increase in reactive nitrogen species.

Solvent Fractionation: It is a separation process of a mixture's constituents. In this study, it was used to separate the bioactive compounds from flavonoids using the solvents n-hexane and ethyl alcohol.

Total Flavonoid Content Assay: It is commonly measured by the aluminum chloride colorimetric assay that compares the results to a flavonoid standard and refers to the assay that measures the total flavonoid content obtained in the study (Shraim, 2021).

Total Phenolic Content Assay: It is commonly measured by Folin Ciocalteu colorimetric assay, which compares the results to a flavonoid standard and refers to the assay that measures the total flavonoid content obtained in the study (Shraim, 2021).

Voacanga globosa: It is an endemic plant found in the Philippines and refers to the plant used in the study.

CHAPTER 2

Review of Related Literature and Studies

This chapter reviews the relevant literature and studies identified through an extensive and comprehensive search conducted by the researchers. Various literature and articles, both local and international were examined to establish a robust foundation for the current study.

Botanical Description of *Voacanga globosa*

The plant *Voacanga globosa* (Blanco) Merr. known as "Bayag-usa" locally is part of the Apocynaceae family and is endemic to the Philippines. Traditionally, bayag-usa has been used to treat cancer, infections, and ulcers (Manzano et al., 2024). Bayag-usa tree can grow as tall as 3 meters. Leaves are simple, opposite, and oblong-elliptic in shape, with a dark green upper surface and a pale underside. Its flowers are noticeable and creamy white. The fruit appears in twos, is brownish, and ranges from 3 to 5 centimeters in diameter. The seeds are embedded in the pulp (Stuart, 2024).

Phytochemical Studies on the Genus *Voacanga*

Voacanga (family Apocynaceae) is a small taxon with 12 species that are found mainly in tropical countries such as Africa, Malaysia, New Guinea, Australia and Philippines. It is closely related to the Tabernaemontana genus, which are commonly found in tropical places in Brazil. Several species within the genus *Voacanga* are particularly noted for their traditional medicinal use (Anand, 2020).

The two endemic species in the Philippines, *Voacanga globosa* (commonly known as Bayag-usa) and *Voacanga megacarpa* (Bayag-Aso) have shown promising pharmacological potential. It has been found in research that Voacanga globosa have anticancer, antimicrobial, and anticholinesterase activity. Although Voacanga megacarpa research, to date, remains at an infancy level, initial studies show that the two species have similar phytochemical compositions (Stuart, 2024). Initial phytochemical screening of the leaves of Voacanga globosa showed the presence of alkaloids, saponins, 2-deoxy sugars, and hydrolyzable tannins. Later bioassay-directed purification led to the isolation of a new spirobisindole alkaloid, globospiramine containing an Aspidosperma-Aspidosperma skeleton. Isolation of other compounds such as deoxyvobtusine, vobtusine lactone, and lupeol also occurred along with globospiramine. (Manzano, 2024).

Voacanga species like Voacanga africana are found to be a source of highly therapeutically valuable bioactive indole alkaloids. Voacanga species have been found to contain indole alkaloids possessing analgesic, antimicrobial, anti-ulcer, cytotoxic, and antioxidant activities. Voacanga indole alkaloids have also prompted the construction of many lead drug candidates due to their unprecedented skeleton structures as well as bioactivities. Notably, Voacanga is the plant genus where ibogaine—a compound used in the treatment of opioid and other chemical addictions—was first isolated.

Furthermore, Voacanga species are a primary source of tabersonine, a primary precursor to the aspidosperma-type monoterpene indole alkaloids (MIAs) synthesis, which exhibit pharmacological activities including antioxidant, hypotensive, and analgesic activities (Cuello et al., 2022). Specific biologically active indole alkaloids that have been obtained from this genus include voafolidine, vocangine, and vobtusine of V. africana, and globospiramine of V. globosa (Coon, 2023).

Additionally, the antioxidant and anti-inflammatory actions of the Voacanga genus have been mostly attributed to their flavonoidal content. Phenolics of plants are one of the significant groups of compounds that are free radical scavengers

or primary antioxidants. (Owayaluja et al., 2019). Antimicrobial activity in Voacanga africana was present in a diverse combination of secondary metabolites including flavonoids, polyphenols, alkaloids, and quinones. The presence of such compounds bears great promise for multifaceted pharmacological activities. Flavonoids and phenolic acids, in particular, are already well known to possess antioxidant and antibacterial properties, which are most probably the reason for the medicinal activity of Voacanga africana (Jampou et al., 2022).

Voacanga, thus, is a valuable source of biologically active metabolites, and the indole alkaloids, polyphenols, and flavonoids are the most impressive among them. *Voacanga globosa* and *Voacanga africana* are some of the species that possess good pharmacological potential. The antioxidant and anti-inflammatory activity of their flavonoid constituents further render them therapeutic potential. More research on the phytochemicals and biological activity of Voacanga species should be done so that they can be utilized best medicinally and new drugs can be prepared.

Phytochemical Studies on *Voacanga globosa*

Voacanga globosa is a Philippines indigenous species which occurs only in Bataan. Fruit and leaf have been traditionally used for ages for the treatment of ulcers, wounds, and food poisoning by traditional medicine. Its most significant medicinal application is because of anticancer activity enabled by biologically active molecules of spiroindole alkaloids consisting of particularly deoxyvobtusine, vobtusine lactone, and globospiramine.

These alkaloids were reported to display cytotoxicity against Mycobacterium tuberculosis and HIV, as well as anticholinesterase activity. Scientific studies of spirobisindole alkaloids, in particular globospiramine, provide proof supportive of the traditional use of the plant against HIV, cancer, and tuberculosis, as attested by in vitro cytotoxicity bioassays (Manzano et al., 2024).

Voacanga globosa extracts were further screened for antimicrobial activity. Disc diffusion identified them to be highly antibacterial against a test panel of microorganisms like *Pseudomonas aeruginosa, Bacillus cereus, Salmonella typhimurium, Staphylococcus aureus, and Micrococcus luteus.* The extract was antifungal against *Candida albicans* and antiprotozoal against *Trichomonas vaginalis* and *Entamoeba histolytica* with potency comparable to metronidazole.

Apoptotic-like protozoal changes were evidenced by fluorescence-based detection. Phytochemical screening established the existence of saponins, alkaloids, 2-deoxysugars, and hydrolysable tannins, validating its use as an antimicrobial treatment and its promise as a source of novel therapeutic compounds (Vital & Rivera, 2011).

Conversely, a study conducted by Guzman et al. (2013) reported that mice treated with a 5 mg/kg V. globosa dose yielded a profound pro-inflammatory response characterized by heightened swelling, white blood cell infiltration, and increased tissue size. Notably, higher doses led to diminished inflammation, indicating that at higher doses, the plant has immunosuppressive properties. Thus, the study suggests that the leaf extract of V. globosa exerts a biphasic (two-phase) effect on inflammation: it acts as an immunostimulant at low concentrations, thereby enhancing the immune response, and as an immunosuppressant at higher concentrations, attenuating the immune response. These findings further validate the ethnopharmacological use of V. globosa in the treatment of boils.

Researchers have identified a new compound named globospiramine along with other alkaloids namely, deoxyvobtusine, deoxyvobtusine lactone, vobtusine lactone and lupeol from the plant *Voacanga globosa*,through the application of advanced spectroscopy techniques, the structure and stereochemistry of globospiramine were

determined, proposing a novel pathway for its unique chemical structure. Globospiramine demonstrated significant activity against *Mycobacterium tuberculosis* (H37Rv strain), and several of these compounds effectively inhibited the enzyme butyrylcholinesterase, particularly deoxyvobtusine. Globospiramine shows strong in vitro activity against tuberculosis while deoxyvobtusine demonstrates significant inhibition of butyrylcholinesterase (BChE). This study increases the diversity of alkaloid structures known to possess both antimycobacterial and anticholinesterase activities, specifically excluding flavonoids. (Macabeo et. al, 2011)

Consequently, researchers have also analyzed the cytotoxic and antimitotic properties of alkaloids extracted from the leaves of Bayag-Usa, wherein they partially purified alkaloids from the plant's leaf extract and tested its biological effects. With the use of FTIR (Fourier Transform Infrared Spectroscopy), they confirmed the presence of alkaloids in the leaf extract. Cytotoxicity was evaluated using the Brine Shrimp Lethality Assay, with artificial seawater as the negative control and 2% ethyl alcohol as the positive control. To assess antimitotic effects, the Allium Test was conducted, measuring the inhibition of cell division in onion root cells, with distilled water serving as the negative control and maleic hydrazide as the positive control. The extract was tested at four concentrations: 1000 ppm, 100 ppm, 10 ppm, and 1 ppm. The outcome was shown to have a statistically significant effect at higher concentrations, namely 1000 ppm. Cytotoxicity was greatest at the Brine Shrimp Lethality Assay, at 1000 ppm, where LC50 (lethal concentration for 50% brine shrimp) was 187.14, which implies very high toxicity. The Allium Test also established that 1000 ppm was the highest to cause antimitotic activity with an EC50 value of 555.67. Overall, this study establishes that higher concentrations of Voacanga globosa extract are more cytotoxic and antimitotic (Lorenzo et al., 2014).

Traditionally, Voacanga globosa had also been employed by Bataan indigenous folks in the healing of ulcers, wounds, and tumors. In the work of Acebedo et al., (2014), ethanol had been utilized for the extraction of V. globosa leaves and subsequently partitioned using hexane and ethyl acetate. MP1, MP2, and MP3 fractions were then isolated through silica gel column chromatography, then high-performance liquid chromatography (HPLC) was utilized. The cytotoxic activities of the fractions were determined by the MTT assay, in which they were tested against the cancer cell lines human colorectal carcinoma (HCT116) and adenocarcinoma human alveolar basal epithelial cells (A549), and the non-cancerous AA8 Chinese hamster ovarian cell line. The MP2 and MP3 fractions were very cytotoxic to the cancer cell lines, whereas the MP1 fraction was not significantly active. In particular, the MP3 fraction caused human colorectal carcinoma cell morphological changes typical of apoptosis, including apoptotic body formation and DNA condensation. TUNEL assays established a time-dependent increase in fragmentation of DNA significantly. The MP3 fraction also caused destabilization of mitochondrial membranes, a necessary step in activation of apoptosis. The occurrence of terpenoids and saponins was confirmed by qualitative phytochemical screening of MP3 fraction.

In conclusion, the results indicate that the MP3 fraction exerts cytotoxic effects on HCT116 cells by triggering apoptosis through the disruption of mitochondrial membrane potential, a vital factor for cell survival.

In a more recent study, the researchers examined how certain alkaloids specifically globospiramine, deoxyvobtusine, and vobtusine lactone, can inhibit HIV replication by affecting the transcription of HIV genes. Since effective HIV transcription is key to viral replication, researchers focused on these alkaloids' effects on the HIV-activated cell lines OM10.1 and J-Lat. They found that globospiramine, at non-toxic levels, effectively blocked HIV replication induced by TNF-α (a protein that promotes inflammation and can activate latent HIV).

Additional testing showed that globospiramine acts on the NF-κB pathway, one of the important signal pathways responsible for HIV transcription. Molecular docking analysis demonstrated that globospiramine has a high affinity for important sites in the NF-κB pathway, specifically the S281 site of the p65 subunit and the activation loop of IKKα, both which are important for NF-κB activation. This tight binding suggests that globospiramine may be an excellent lead for the design of novel alkaloid-derived anti-HIV drugs (De Jesus et al., 2022). In addition, laboratory tests proved that globospiramine reduces the viability of Candida cells in a concentration- and time-dependent manner, killing about half of the C. albicans cells within 60 minutes. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) tests indicated that globospiramine has both inhibitory and fungicidal activity against C. albicans, and a weaker growth-suppressing activity against C. tropicalis. Further experiments indicated that globospiramine activates cell death-linked enzymes in C. albicans. Molecular modeling suggested that globospiramine most probably interacts efficiently with enzymes like 1,3-β-glucan synthase and Als3 adhesin, which play roles in Candida development and adhesion, and these have been suggested as targets for its antifungal action (Manzano et al., 2024).

As per Manzano et al. (2024), three alkaloids' antiproliferative and cytotoxic activity were evaluated through in vitro models, including MTT and CellTiter Blue assays, in different cancer cell lines like A431,MCF-7, KB3.1, L929, A549, SKOV-3 and PC-3. The globospiramine had the greatest activity and additional investigation with Western blotting identified that globospiramine led to increased cleaved caspase-3 and PARP, proteins that are markers of apoptosis (programmed cell death) in cancer cells. This indicates that globospiramine can induce cancer cell death by the mechanism of apoptosis.

In conclusion, the study presents evidence that spirobisindole alkaloids, especially globospiramine, hold promise as anticancer agents, highlighting its capacity to induce apoptosis in cancer cells.

Flavonoids and their Biological Activity

Polyphenols are a class of bioactive compounds possessing multiple biological effects. They are regarded as secondary products of plant metabolism existing in different parts of plant. Vascular plants Flavonoids are group of bioactive phenolic compounds of lower molecular weight and are present in photosynthesizing cells (angiophores) cells (Karak, 2019). These compounds are characterized by a benzopyrone ring bearing a number of polyphenolic groups at different positions. Abundant in nature, they are found in an enormous variety of plant foods, including fruits, vegetables, herbs, nuts, seeds, flowers, and leaves. It is present in high amount initially at diverse sites and also found in wide variety of calibration sources such as citrus fruits, berries, apples, onions, broccoli, tea etc. They exist in abundance in many parts of plants, where they have become important in many foods that contribute to the known antioxidant and anti-inflammatory activities in plant-based diets. Combination of these different parts of plant collectively have participated toward its medicinal properties and biological activity owing to the existence of active phytochemical moieties (Ullah et al., 2020).

These compounds have caused an abundance of intrigue due to their multifaceted nature and impacts on human and animal health and the widespread existence in the plant kingdom. Flavonoids influenced significantly to effective medicinal procedures in the past as well as of today. Flavonoids exhibit a variety of biological activities, most notably their antioxidant properties, which have been associated with the prevention and management of a range of diseases, including Alzheimer’s disease, cancer, and heart disease. These compounds help mitigate oxidative stress and inflammation, key factors in the development and progression of these chronic conditions. As a result, flavonoids are

considered promising candidates for supporting health and reducing the risk of diseases linked to oxidative damage and cellular dysfunction. Eight to ten flavonoids are connected, having a wide range of benefits for health and are essential parts of many different pharmaceutical, medical, nutraceutical, and cosmetic applications. It is as a result of their strong anti-inflammatory, antioxidative, antimutagenic, antibacterial, anti-carcinogenic, vascular activities, the capacity to scavenge free radicals and their ability with other therapeutic qualities combined to regulate vital cellular enzymes (Karak, 2019).

While flavonoids possess several beneficial properties, their ability to neutralize free radicals and function as antioxidants is undoubtedly their most significant feature. The kind of functional group and how it is arranged around the nuclear structure determine the antioxidant potential within flavonoid classes. The ability of flavonoids to neutralize free radicals depends on the specific arrangement and positioning of hydroxyl groups on the catechol B-ring and the pyran C-ring. The resonance structure of flavonoids allows the hydroxyl group to donate both an electron and a hydrogen atom to a free radical, stabilizing it and creating a relatively stable flavonoid radical in the process. This molecular interaction is a key to the antioxidant action of flavonoids. Their antioxidant effects are multifaceted as they directly scavenge reactive oxygen species (ROS), reduce the formation of ROS by chelating metal ions that catalyze oxidative reactions, inhibit enzymes involved in free radical production, and enhance the activity of endogenous antioxidant systems. In this way, flavonoids contribute to protecting cells from oxidative damage and prevent diseases related to oxidative stress. Most flavonoids are glycosides, and their antioxidant power is determined by the number and position of their sugar linkages. Aglycones are less abundant but are more potent antioxidant compounds (Dias et al., 2021).

Flavonoids can serve as cardioprotective agents via anti-inflammation, antioxidation, vasodilation, and anti-apoptosis in the endothelium. Besides being heart healthy, these compounds have antimicrobial properties that work against bacteria in more than one way. They might permeate bacterial membranes, perturbate the structure of lipid bilayers and prevent the biofilm formation. Flavonoids disrupt other bacterial processes including the formation of cell envelopes, nucleic acids, the electron transport chain, and ATP synthesis. In addition, flavonoids have antifungal activity through other mechanisms, such as the effect on the plasma membrane, their influence on the mitochondria dysfunction, and blocking the synthesis of cell walls, cell division, RNA, and protein biosynthesis. By limiting ROS species, lowering lipid peroxidation, and preventing membrane disruption, apigenin and baicalein can function as antifungals. It also possesses antiviral action by inhibiting the binding and entry of viruses into cells, disrupting the process of viral reproduction or translation, and stops the virus from escaping the cell (Dias et al., 2021).

Flavonoids lower oxidative stress in epilepsy and have neuroprotective properties. Many flavonoids display anticonvulsant properties in the central nervous system by interacting with the benzodiazepine site on the y-Aminobutyric acid Type A (GABAA) receptor, thereby modulating its activity and enhancing inhibitory neurotransmission. Consuming berry flavonoids helps older adults remember things better. Dietary cocoa flavanols enhance dentate gyrus function, which benefits older people's cognitive function. Intake of cocoa flavonols enhances cognitive function and prevents several forms of cognitive decline. In healthy people. Consumption of this improves cerebral cortical oxygenation and cognitive function. The primary source of oxidative stress, ROS, are also implicated in the etiology of a number of neurological conditions. Increased ROS causes lipid peroxidation, protein oxidation, and DNA damage, all of which cause neuronal cells to undergo apoptosis. Utilizing antioxidants, including flavonoids, may

help lessen the toxicity of oxygen-free radicals. These flavonoids may be able to reduce the pathophysiology of a number of neurological conditions and combat the toxicity of oxidative stress. Phytochemicals called flavonoids have been shown in numerous studies to have a beneficial influence on neurological conditions. Beneficial effects on the cellular stress response have been demonstrated by flavonoids. According to a number of studies, these flavonoids may be effective for neurological conditions (Devi et al., 2021)

All flavonoids exhibit a C=O stretching band around 1650-1560 cm-1. Ring C-C stretching vibrations are observed in most flavonoids (except flavanols) between 1560-1465 cm-1. Other key spectral regions, such as those associated with C-C stretching in specific groups (e.g., 1475-1400 cm-1 for flavonols and anthocyanins), C-O stretching, and C-H bending, provide valuable information for flavonoid classification. While some bands are common across groups, others, like those specific to flavonols (1570-1545 cm-1, 1255-1235 cm-1, 1140-1125 cm-1, 1095-1075 cm-1) or flavanols (1135-1120 cm-1), can aid in their identification. Bands below 970 cm-1 are unique to each group but primarily by their assignment, not their exact position (Kryza et.al., 2022).

By means of this, numerous reports have indicated that flavonoids offer an extensive array of health advantages.

Preclinical research conducted on mouse models has overwhelmingly demonstrated the therapeutic effects of flavonoids. In order to avoid compromising the absorption and bioavailability of flavonoids, distinct methodologies ought to be employed in clinical trials. Enzymes from their biosynthetic pathways should be expressed in other plants and species that grow quickly in order to boost their production. Moreover, flavonoids conjugates with other significant medications may increase the potency of such substances. (Ullah et al., 2020)

Flavonoids in the Development of Neurodegenerative Disorders

Numerous epidemiological evidence suggests that a diet rich in flavonoids can enhance cognitive function and help prevent age-related neurodegenerative decline (Godos et.al., 2020). Flavonoids have garnered increasing attention.

Consequently, developing anti-aging medications based on flavonoids is crucial for enhancing the quality of life of elder populations and slowing the aging process and improving the quality of life of elderly individuals (Pacularu-Burada et.al., 2019). This capacity of flavonoids is due to their chemical properties and broad spectrum of biological actions.

Lamptey et. al (2022) highlighted that one of the major challenges in identifying new treatments for neurodegenerative diseases is to identify compounds that can cross the blood-brain barrier (BBB) without disrupting its integrity, as these have been promising in stopping or reversing neurodegeneration. Hasan et. al (2023), an in vitro model using rat and human brain cells from the endothelium has shown that the pharmacokinetic profile of the flavonoids makes them capable of crossing this barrier which is critical towards understanding their ability to transmit therapeutic effect to the brain. Hasan et.al (2023) also reported that also reported that flavonoids like Naringenin and its in vivo metabolites showed more ease of passing through the BBB while Quercetin was found to interact with p-glycoproteins related to Alzheimer's, Parkinson's disease, and frontotemporal dementia. Also, a study conducted by Hajialyani et.al. (2019) emphasizes the flavonoid glycoside, Hesperidin, can exert its improved neuroprotective properties by the alleviation oxidative stress and neurotrophic growth factors in the CNS since it has the ability to cross the blood-brain barrier to reach the brain. The research further suggests that these processes could be universalized to other flavonoids of similar

nature. These studies support that an important property of flavonoids that contributes to their systemic therapeutic effects is their ability to cross the BBB, making them able to target and safeguard brain cells as well as fight neurodegenerative diseases. The action mechanisms of flavonoids responsible for their possible anti-neurodegenerative effects have also been the subject of recent research. Neurodegeneration is mainly fueled by a number of cellular and molecular players, such as oxidative stress, protein aggregate accumulation, neuroinflammation, dysfunctional mitochondrial function, apoptosis, and alteration in autophagy. Polyphenols and flavonoids are considered promising neuroprotective agents because they can modulate and influence these key cellular events that are associated with neurodegeneration.

One characteristic of the pathogenesis of neurodegenerative diseases, particularly Alzheimer's disease, is the development of senile plaques due to the progressive accumulation of the beta-amyloid (Aβ) in the brain (Ali et.al., 2019).

A variety of research work has proved flavonoids exhibit anti-amyloidogenic properties, preventing formation of Aβ aggregates and its neurotoxic effects when these accumulate. Notably, the in-vivo study by Nakajima and Ohizumi (2019) explored whether Nobiletin, a flavone from the Citrus spp., with multiple polyethoxy groups, may be used in the alleviation of memory deficiencies through the inhibition of Aβ-infused rat models. Their results indicate that there is a dramatic reduction in intracellular and extracellular Aβ levels following nobiletin treatment. This was attained through the induction of neprilysin, the key protease for the physiological degradation of Aβ in the brain, thus augmenting Aβ degradation that mimics the progression of Alzheimer's Disease.

Neuroinflammation also significantly contributes to the progression of oxidative-stress induced neurodegenerative disorders like Alzheimer’s disease, Amyotrophic Lateral Sclerosis, Parkinson’s disease and Huntington’s disease (Zhang, et.al. 2023). The researchers reported that this is marked by microglial activation which leads to the secretion of pro-inflammatory cytokines, initiating a cascade of events ultimately contributing to the gradual death of neuronal cells. Chronic inflammation arises when the immune cells are unable to alleviate inflammation, resulting in toxicity and enhances aggregation of proteins. Extensive studies have demonstrated that flavonoid compounds reduce neuroinflammation by suppressing pro-inflammatory mediators, promoting the release of anti-inflammatory agents, and influencing microglia and astrocytes polarization. Chen et.al (2022) summarized the mechanisms by which several of natural flavonoids work against neuroinflammation. The study included flavonoids such as luteolin, nobiletin, diosmetin, baicalin, kaempferol, morin, isovitexin, quercetin, rutin, apigenin, myricetin, hyperoside and many others, exerting their effect through the inhibition of pathways like NF-KB, MAPKs, JAK/STAT pathways, and NLRP3 activation, all which promote inflammation. Additionally, flavonoids activate beneficial pathways, including AMPK, Nrf2, CREB/BDNF, Wnt/β-Catenin, and PI3K/Akt, HMGB1 deaceylation. These actions contribute to their neuroprotective effects by reducing inflammation and supporting neuronal health through cellular repair.

Another mechanism by which flavonoids exert their effect against neurodegenerative disorders is the chelation of heavy metals in the brain. Research has previously demonstrated that imbalances of metal ions in the brain participate in the development of Alzheimer’s and Parkinson’s Disease. This is closely tied to the aggregation of Aβ proteins and hyperphosphorylation of tau due to abnormal amounts of iron, zinc, copper and calcium in various areas of the brain (Ijomone et.al., 2024). Consequently, there is a need for metal-chelating compounds to resolve the pathology caused by this imbalance. Rodrigez-Arce & Saldias (2021) investigated rutin, morin, and quercetin as they were found to exhibit

strong chelating abilities for metal ions. Binding to metal ions help mitigate both oxidative stress and reduce the overload of neurotoxic metals implicated in neurodegenerative disorders.

In connection to the mechanisms mentioned above, oxidative stress underscores most of these harmful effects on the brain and CNS leading to neuronal damage which is the main pathology of most neurodegenerative disorders. This occurs due to the imbalance between ROS and RNS production, and the antioxidative defense of the body. Various studies have already established the biological role of flavonoids as antioxidants through free-radical scavenging. Kim et.al. (2019) isolated quercitrin, isoquercitrin, and afzelin from the *Acer okamotoanum* ethyl acetate fraction and observed the antioxidative effects of these three flavonoids through in vitro investigation using SH-SY5Y human neuroblastomal cells. Results have shown that the isolated flavonoids protect against cellular oxidative stress through ROS-scavenging, regulation of inflammation, and apoptosis. Thirukumaran et.al. (2024), also evaluated the ability of myricetin to prevent free radicals formation and the likelihood of neurodegeneration through in vitro methods. The results indicate myricetin has the ability to directly neutralize ROS and can increase the efficacy of antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase, along with the ability to reduce amyloid-beta (Aβ) aggregation and inhibit acetylcholinesterase. In interpreting these results, it shows that flavonoids have extensive potential to target oxidative stress and neurodegeneration.

Finally, Acetylcholinesterase (AChE) contributes to neurodegenerative disease pathogenesis via modulation of cell death, oxidative stress, toxic protein aggregation, and inflammation (Walczak-Nowicka & Herbet, 2021). Thus, inhibition of AChE has been the subject of treatment of some neurodegenerative disorders. Flavonoids are found to exhibit significant AChE inhibitory activity in prior research. In a study by Zhou and Hua (2020), the AChE binding activity of different kaempferol glycosides was established through molecular docking analysis. Platycladus orientalis kaempferol rhamnoside was discovered to possess high activity against acetylcholinesterase. But its AChE binding activity was reduced due to the occurrence of steric hindrance in its structure. In addition, Cuong et.al (2023), explored four flavonoids isolated from *Pinus krempfii* were explored to identify their capacity to inhibit AChE and BChE, the enzymes implicated in Alzheimer's disease. Tectochrysin and galangin, in particular, were found to possess significant inhibitory activity and remarkable binding affinity to the target enzymes of interest, making them accessible for use as neuroprotective agents.

Rhoifolin is a flavonoid glycoside, and it possesses diverse biological activities with extensive enzyme inhibitory and antioxidant activities. It has been established that rhoifolin possesses moderate inhibition against butyrylcholinesterase (BChE), amylase, and tyrosinase. Rhoifolin inhibited BChE with 4.03 mg GALAE/g and tyrosinase inhibition with 7.44 mg KAE/g with amylase activity as well. Interestingly, this is the first reported case where isolated or pure rhoifolin was tested for its enzyme inhibitory activity, different from the previous studies on rhoifolin-rich plant extracts. Yet, while presenting these moderate enzyme inhibitions, Rhoifolin also demonstrated weak antiradical activities with the following values of 1.04 mg TE/g (in DPPH) and 0.43 mg TE/g (in ABTS), and weak reducing capacities in the CUPRAC (16.79 mg TE/g) and FRAP(10.29 mg TE/g) tests. The weak antioxidant activity is thought to be due to the glycosylation at the 7-OH position on the flavanone skeleton. Despite this, even these weak antioxidant properties may still hold potential in synergistic applications or within specific biological contexts where even subtle antioxidant effects can contribute to overall protective mechanisms.

Oxidative Stress-Induced Neurodegenerative Disease

Major percentage of neurological diseases that are pertinent to public health are neurodegenerative disorders. Numerous general and disease-specific variables interact intricately in the pathophysiology of neurodegenerative illnesses, culminating in neuronal loss and degeneration and the subsequent clinical manifestations. An imbalance between pro-oxidant species and antioxidants causes oxidative stress, which is characterized by a decrease in endogenous antioxidant levels and an increase in reactive oxygen and reactive nitrogen species (Olufunmilayo et al., 2023). There is no known cure or treatment for the most prevalent neurodegenerative diseases, which include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Memory and cognitive impairments, and speech and movement disorders are typical presentations of these diseases (Rong et al., 2020). Neurodegenerative is triggered by the aggregation of some proteins. These include huntingtin in HD, a α-synuclein in PD, β-amyloid plaque and tau in AD, and the inclusion of FUS in ALS, as well as superoxide dismutase 1 (SOD1) and transactivation response (TAR) DNA-binding proteins (TDP)-43. Proteotoxic stress, impaired function of the ubiquitin-proteasomal and autophagosmal/lysossomal pathways, mitochondrial dysfunction, glutamate toxicity, calcium overload, neuroinflammation, and the effects of aging all contribute to the progressive degeneration and death of neurons in neurodegenerative diseases. These factors collectively lead to the breakdown of cellular homeostasis and neuronal integrity, accelerating disease progression (Hassan et al., 2022)

Oxidative stress and mitochondrial dysfunction are important factors in the emergence of neurodegenerative disorders. The primary location for metabolic processes where electrons are ultimately moved via the electron transport chain is the mitochondria. Reactive oxygen species, such as superoxide anion radicals (O-2), are produced in greater quantities when mitochondrial function is compromised. Glutathione peroxidase (GPx) breaks down hydrogen peroxide, which is produced by mitochondrial superoxide dismutase. Overproduction of ROS results is apoptosis induction, ATP loss, a drop in mitochondrial membrane potential, and consequent cell death. In addition, ROS causes DNA damage, lipid peroxidation and the production of protein carbonyl molecules. Accordingly, AD, PD, HD, and ALS are all accelerated by oxidative damage, either directly of indirectly (Hassan et al., 2022) Mitochondrial ROS are especially harmful to mitochondrial DNA (mtDNA), which is more susceptible to mutation and lacks histone protection. The function of postmitotic neurons is greatly impaired by the frequent damage to mtDNA, hence mitophagy is essential for removing damaged mitochondria. Mitophagy is the breakdown of mitochondria brought on by cues such as oxidative stress or hunger. While oxidative stress can trigger mitophagy by lowering the potential of the mitochondrial membrane, it can also hinder it by interacting with its regulators. ROS can S-nitrosylate parkin, a crucial regulator of mitophagy implicated in Parkinson's disease, which inhibits it and stops damaged mitochondria from being removed. A working theory of aging that integrates ROS, DNA damage, and mitochondrial theories has been developed as a result of the essential role that mitochondria play in the production of ROS. According to the notion, DNA damage triggers the activation of kinases and PARP, which lowers the levels of NAD+, a donor in the synthesis of ATP and a cofactor for numerous metabolic processes. Reduced NAD+ causes mitochondria to pair in an effort to satisfy high energy demands since it increases the need for oxygen consumption and ATP generation. Further DNA damage is caused by mitochondrial coupling, which also raises free radicals, lowers mitophagy, and increases membrane potential. The idea that mitochondria is important is aging and related neurological illnesses is supported by the intimate connection between dangerous ROS and mitochondria (Tucker et al., 2021)

An essential part of the respiratory system, oxygen is necessary for an organism to survive. Oxidative stress arises from an excess of free radicals and reactive oxygen species (ROS) produced from molecular oxygen. Although mitochondria are central to both oxygen consumption and ROS generation, the cell's homeostatic mechanisms normally regulate ROS levels to maintain balance. However, when this balance is disrupted, it can lead to neuronal dysfunction, apoptosis, or necrosis, all of which are prominent features of neurodegenerative diseases. The brain, as teh body's most metabolically active organ, is highly susceptible to oxidative damage. It consumes about 20% of the body's total oxygen, making it particularly vulnerable to the harmful effects of oxidative stress. The enhanced oxygen demand, coupled with somewhat restricted antioxidant defenses, increases the likelihood of damage and degeneration of neurons in neurodegenerative disorders such as Alzheimer's and Parkinson's and other related disorders. Moreover, redox-active metals iron and copper at high concentrations in the brain are implicated in the production of ROS, again inducing oxidative stress and neuronal injury. Because of their PUFA concentration, cell membranes of brain cells are extremely sensitive to lipid peroxidation. Nevertheless, an antioxidant, glutathione, is extremely important for detoxifying ROS species in brain cells when it is available in sufficient amount. Neurodegenerative diseases such as AD, PD, HD, and Machado-Joseph disease are induced by the elevation of ROS and the decline in GSH in the brain (Singh et al., 2019).

Antioxidants

Phytochemicals act as vital nutrients and dietary fiber, protecting the body from many diseases. Phytochemicals are synthesized into secondary metabolites, which are formed in small amounts in higher plants. Some of the common phytochemicals are steroids, alkaloids, flavonoids, tannins, terpenoids, and so on. Several phytochemicals, such as flavonoids, possess antioxidative activities that prevent many diseases. Antioxidants have a significant role in preserving cellular integrity by neutralizing free radicals, which, if not controlled, may cause cellular damage and lead to the pathogenesis of diseases, such as cancer and cardiovascular diseases. Antioxidants are generated naturally by our body, but their production declines with age and is perhaps too weak when there is an overproduction of free radicals.

Plants from the Apocynaceaea family are potent sources of antioxidants such as carotene, flavonoids, and phenolics.

The entire plant family can be exploited for their antioxidative use (Yadav et al., 2024).

Oxidative stress is recognized as a critical contributor to neurodegenerative diseases such as Alzheimer's, Parkinson's, and ALS, as well as to conditions associated with nerve damage, including stroke, peripheral nerve injury (PNI), and spinal cord injury (SCI).

In the presence of oxidative stress, malfunctioning mitochondria produce free radicals while depleting the energy necessary for cellular operations, thereby triggering inflammation and ultimately leading to cell death. There is debate on whether oxidative stress causes mitochondrial dysfunction or vice versa. However, antioxidants are thought to help restore redox balance, reduce mitochondrial dysfunction, and slow degeneration. Antioxidants mitigate oxidative stress by neutralizing free radicals and inhibiting oxidative chain reactions. These include endogenous antioxidant enzymes, nonenzymatic antioxidants, and both natural and synthetic external sources, all of which contribute to the preservation of redox balance (Ashok et al., 2022).

Role of Antioxidants in Neurodegenerative Disease Development

The typical sign of aging is a biological inability to sustain proper homeostasis, which results in a disturbed balance between the endogenous antioxidant system and the release of prooxidants, a continuing inflammatory process, and an increased likelihood of neurodegenerative diseases. Neurodegenerative diseases associated with aging, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and epilepsy, have a sophisticated genetic origin with various causes. Moreover, a common element in the progression of numerous disorders is elevated oxidative stress (Tchekalarova & Tzoneva, 2022).

The normal functioning human brain uses about 20% of the total basal oxygen (O2) and gets 15% of the cardiac output. According to the theory that neurodegeneration has a mitochondrial origin, oxidative tissues that require a lot of energy are particularly susceptible to abnormalities in the oxidative phosphorylation system (OXPHOS). Additionally, the brain's most actively metabolizing regions—the cortex, specifically the motor cortex and thalami—are also the most susceptible to hypoxia ischemic encephalopathy. Accordingly, reducing free ROS is seen as a possible method of restoring redox balance in the management of several brain disorders through antioxidant therapy techniques (Morén et al., 2022).

Certain characteristics are presented by numerous chronic neurodegenerative diseases of the CNS, including oxidative stress, inflammation, synapse dysfunction, protein misfolding, and impaired autophagia. Along with other forms of reactive oxygen species, neuroinflammation can also entail mast cell activation, which contributes to oxidative stress.

ROS and free radicals can be effectively neutralized by antioxidants, reducing the damage created by oxidation.

Epigenetic modifications can decrease the expression of antioxidant genes, such as the manganese superoxide dismutase enzyme, increasing oxidative stress in tissue. On the other hand, damage from free radicals can change DNA.

The epigenetic landscape of genes may alter antioxidant activity and contribute to neurodegenerative illness. Reactive oxygen species that damage cells are increased by the disparity among the generation of free radicals and antioxidant action (Houldsworth, 2023).

Antioxidant therapies have demonstrated positive effects in representation of AD, including elevated ATP and SOD, decreased Apolipoprotein E and Aβ fragments, and enhanced hippocampus synaptic plasticity. It has also been demonstrated that mushrooms like *Hericium erinaceus*, carotenoids, and polyphenols have anti-inflammatory and qualities that protect brain functions.

Vitamins E and C, CoQ10, and ALA supplementation have been demonstrated to enhance behavioral variables, decrease dopaminergic cell death, and restore corticostriatal synaptic plasticity in Parkinson's disease (PD). It has been demonstrated that other antioxidants, like crocin or fucoxanthin, block autophagy and enhance homeostasis, mitochondrial enzyme activity, and changes in behavior.

Mutations in the SOD1 genes cause ALS by decreasing the activity of antioxidant enzymes, which renders them useless in reducing ROS levels. It has been demonstrated that curcumin reduces amyloidogenicity and neurotoxicity by preventing SOD1 amyloid fibrils from aggregating and fibrillating. It has been discovered that using strawberry

extracts high in anthocyanins can enhance the ability to grip, prevent spinal motor neuron death, postpone the development of disease, and maintain neuromuscular connections (Ashok et al., 2022).

A lack of balance between pro- and antioxidant species is known as oxidative stress, which damages molecules and cells. It significantly influences the development of age-related disorders like neurodegenerative diseases. By the production of prooxidants or stopping their proliferation, antioxidants can prevent autoxidation, which in turn lowers oxidative stress, boosts immunity, and promotes healthy longevity (Pisoschi et. al., 2020).

Antioxidant Property and AChE Inhibitory Activity of Flavonoid-Rich Plants

Antioxidant activity and AChE inhibition exert their mechanism of action independently in preventing the progression of neurodegenerative disorders. Still, there is a common impact of their activity that is modulation of oxidative stress.

With that, several research has investigated the dual action and therapeutic potential of flavonoid-bearing plants in combat against neurodegenerative diseases through antioxidant property and inhibitory activity towards AChE.

Ferreira et.al. (2020) investigated the Quercus suber cork and its ethanol-water extract for its activity against Alzheimer's disease by assessing its free radical scavenging activity using DPPH and FRAP assay as well as its anticholinesterase activity. Results indicate a significant correlation between the high content of phenolic and flavonoid in Corkback extracts with antioxidant and AChE inhibitory activities.

Another study conducted by Adetuyi et.al. (2024) on the methanol leaf extracts of Kalanchoe crenata, Spondias mombin, and Carica papaya proved that the isolated compounds from the plants, like quercetin, kaempferol, gallic acid, etc., met the criteria for a potential anti-Alzheimer drug. This is because the high positive correlations between AChE inhibition and activities like DPPH scavenging, nitric oxide (NO) scavenging, and lipid peroxidation inhibition, with r^2 values above 0.7. This suggests that the extracts' compounds that contain antioxidant activity due to high total phenolic and flavonoid contents also potently inhibit AChE.

In a similar way, Diniso et al. (2022) determined the polyphenolic content, free radical scavenging activities, and inhibitory effects of cholinesterase in a methanol extract of Dalbergiella welwitschii leaves and its partitioned fractions (n-hexane, ethyl acetate, and aqueous).

Findings also demonstrate that an increase in the concentration of *Dalbergiella welwitschii*, corresponded to an increase in their ability to inhibit free radicals and cholinesterase enzymes (AChE and BuChE), though this concentration-dependent effect was not observed in lipid peroxidation inhibition. The highest concentrations of polyphenolic compounds, such as flavonoids and phenols, were found in the ethyl acetate fraction.

These findings underscore the therapeutic promise of targeting both oxidative stress and AChE in the treatment of neurodegenerative diseases, as antioxidant and anticholinesterase activity both show parallel effects with one another.

Current AChE Inhibitors in Neurodegenerative Disease Treatment

Despite a century of extensive research, effective treatments that modify the course of neurodegenerative diseases remain lacking, posing a significant threat to society. A well-recognized gap in knowledge and resources has hindered the progression of basic research into viable therapies. Furthermore, the evaluation of vast amounts of preclinical data

remains challenging, limiting the ability to make informed choices on therapeutic technologies and clinical candidates for development. Currently, only a limited number of therapies exist for neurodegenerative diseases, and even these show relatively low success rates, highlighting the need for the clinical and scientific communities to develop new potential treatments (Kumar et al., 2022).

Acetylcholinesterase (AChE) primarily functions to regulate the levels of acetylcholine, a crucial neurotransmitter. This role makes AChE an excellent molecular target for treating neurodegenerative diseases and dementia (such as Alzheimer's disease). Many attempts have been made over the years to inhibit AChE, aiming to create compounds that might slow dementia progression in Alzheimer's disease patients. Patients are often prescribed AChE inhibitors such as donepezil, galantamine, or rivastigmine. These drugs inhibit acetylcholinesterase and are used to manage the symptoms of Alzheimer's disease. By blocking the breakdown of acetylcholine, a neurotransmitter critical for cognitive function, they help enhance cholinergic transmission, which is often deficient in Alzheimer's patients. While all three drugs target acetylcholinesterase, rivastigmine also inhibits butyrylcholinesterase. However, these medications do not alter the disease's progression or reverse its damage. Due to potential side effects, especially gastrointestinal problems and changes in behavior, they are typically prescribed in low doses, gradually increasing as tolerated (Sridhar, 2021). Other compounds, including tacrine and huperzine A, have also been explored for their AChE-inhibiting effects in AD patients. However, existing inhibitors are unable to reverse Alzheimer's disease. These medications are administered solely to slow the progression of the disease. Moreover, patients taking these medications frequently experience side effects like nausea, vomiting, and diarrhea, though these are typically temporary. A major limitation for some patients, however, is the presence of co-morbid conditions such as asthma, heart disease, peptic ulcers, or chronic obstructive pulmonary disease, which are prevalent in older adults and can restrict the use of AChE inhibitors. Research continues to seek new, more effective AChE inhibitors (Bagrowska et al., 2024). More exploration regarding new ACHE inhibitors is necessary considering the limitations of current pharmacological treatments.

Solvent Fractionation

Solvent fractionation or partitioning is typically the preferred technique for eliminating impurities including colors, terpenes, lipids, and wax when phenolic compounds are being obtained from a plant material in its crude form (Alara et. al., 2021). As shown from various studies, fractionation employing the quaternary solvent system hexane-ethyl acetate-methanol-water produced over 60% of the extracted free flavonoids because of its flexibility and wide spectrum of polarity (De Luna et. al., 2020). In extracting phenols, pure alcohol dehydrates and collapses plant cells, breaking down the solute-cell wall link. For this reason, a combination of water and methanol is frequently used as a solvent to extract phenolic chemicals. It is also necessary to incorporate a non-polar solvent like n-hexane to extract lipophilic chemicals from the plant source because such chemicals can alter the outline of the structure of flavonoids and its consequent products (Tzanova et. al., 2020). Furthermore, with the aim of obtaining a greater variety of phytochemicals, fractionating crude extracts with higher polarity utilizes serial exhaustive extraction using first a non-polar solvent, such as hexane, to a more polar solvent (Alara and Abdurahman, 2019). In isolating non-polar flavonoids, ethyl acetate is the ideal solvent to employ because of its high attraction (Dias et. al., 2021). In conducting the fractionation, dried plant samples were extracted consecutively using equal amounts of solvents with escalating polarity over the course of 2 days, while being continuously shaken. The process was conducted three times to get the highest yield of each fraction. To get solid residues of the fractions, the extracts were filtered, pooled appropriately, and then evaporated at 40 C under decreased pressure (Alhawarri et. al., 2021)

DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

Radical scavengers neutralize the damaging effects of reactive oxygen species (ROS), bringing much stability in the process. Inhibiting lipid oxidation and scavenging peroxide radicals are considered to be the most important mechanisms of antioxidants, one which needs to dominate antioxidant determination tests like 2,2-diphenyl-1-picrylhydrazyl (DPPH), N,N-dimethyl-p-phenylenediamine (DMPD), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and $O_2 \cdot^-$, common spectrophotometric assays of antioxidant activity.

The DPPH radical assay evaluates the ability of the antioxidants to be able to scavenge DPPH radicals using spectrophotometry. DPPH is said to be a free radical with a stable, purplish color, due to the presence of an unpaired electron in it, which is capable of absorbing light in the visible spectrum. Free radical, but stable molecule due to resonance stabilization between phenyl and picryl groups. When an antioxidant is added to a DPPH solution, it reacts with the radical. The antioxidant transfers a hydrogen atom, reducing the DPPH radical to its corresponding hydrazine form (DPPH-H). This reaction neutralizes the unpaired electron, turning DPPH into a non-radical species.

The reduction of DPPH is characterized by a change of purple radical to pale yellow hydrazine form. The intensity of color change is directly proportional to the antioxidant property of the substance tested. This color change is then quantified using UV-Vis spectroscopy. (Gülçin and Alwasel, 2023)

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

Despite the antioxidant defense mechanism in humans, cell damage still plays a significant role in the development of diseases associated with oxidative stress. This damage can result from the oxidative modification of essential biological macromolecules, including lipids and proteins. As a result, recent studies have focused on the process of free radical oxidation and the general action of antioxidants (Gülçin and Alwasel, 2023).

Electron Transfer-based assays are a category of methods used to measure the antioxidant of the substance based on its ability to donate electrons to a reactive compound, reducing it to a less reactive form. CUPRAC Assay is a variant of Ferric Reducing Antioxidant Power (FRAP) with the mechanism of electron transfer rather than hydrogen atom transfer (Cerretani, 2010). FRAP Assay is based on the ability of a compound to reduce Fe3+ to Fe2+ while CUPRAC is based on Cu2+ reduction to Cu+.

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay allows Total Antioxidant Capacity (TAC) of hydrophilic and hydrophobic extracts. The CUPRAC assay was found to be a highly effective method of analyzing the antioxidant activities of natural and synthetic molecules. The method is seen to be valid with high cost-effectiveness, high reaction velocities, short analysis durations and stability. These benefits render the CUPRAC assay a potent and efficient method for the quantification of the antioxidant capacity (Akar et al, 2019). The assay reagent (copper (II)-neocuproine (2,9-dimethyl-1,10-phenanthroline, is able to oxidize antioxidants that are yielding a colored product.

CUPRAC assay is based on redox reaction between the reagent and thiol group-containing antioxidants in the sample. CUPRAC reagent itself gets reduced under such conditions to give an orange-yellow copper(i)-neocuproine chelate complex. The complex shows a color change, which is measured by absorbance at 450 nm on a spectrophotometer (G-Bioscience, 2019). CUPRAC treatment will be equivalent to Trolox Equivalent Antioxidant Capacity (TEAC) because of the lowered redox potential and to its pro-oxidation activity. It implies that copper ions engage in only the desired

redox reactions; hence, it is more reliable in measuring antioxidant capacity. The CUPRAC method could also prove to be useful if a compound can cause oxidation under established conditions (Cerretani, 2010).

Acetylcholinesterase

Acetylcholinesterase (AChE) is an important enzyme in the human nervous system, including both its peripheral and central branches. Its primary function is to hydrolyze the neurotransmitter acetylcholine (ACh) into choline and acetic acid ions. The latter enzyme can exist in two major forms. The latter: pure homomeric oligomers refers to variants like monomers, dimers, tetramers etc.[5] These forms are generally soluble inside cells, possibly for secretion or for attachment to the outer membrane via glycerophospholipid bridges. The second form, the more complicated heteromeric AChEs are mainly present in neuronal synapses. This form is usually a tetramer of catalytic subunits, disulfide bonded to a smaller structural subunit that is lipid associated and positioned to the extracellular surface of the cell membrane (Vecchio et al., 2021).

AChE belongs to the serine hydrolase superfamily and is distinguished by a catalytic site located in a spacious hydrophobic gorge. At this site a catalytic triad involving serine, histidine, and glutamic acid is located. The nucleophilicity of the carboxylate group is transferred to the serine hydroxyl by the imidazole ring of histidine. This allows the serine residue to displace choline from the substrate and to produce an acetyl-enzyme intermediate. This intermediate is subsequently hydrolyzed to release acetate ions (Zhao et al., 2024).

Acetylcholinesterase (AChE) is a crucial therapeutic target for the treatment of neurodegenerative diseases with various causes and an irreversible course. The current options of treatment are mainly symptomatic, but they are extremely important in helping with symptoms and improving the quality of life in the patient. Therefore, the search for new therapeutic approaches remains a priority for researchers everywhere (Cañizares-Carmenate et al., 2022).

Acetylcholinesterase (AChE) Inhibition Assay

Neurodegenerative diseases can be symptomatically managed through cholinesterase inhibition. The breakdown of acetylcholine in the normal and healthy brain is most likely caused by acetylcholinesterase (Acero & Amor, 2022). The enzyme acetylcholinesterase is in charge of breaking down acetylcholine, a neurotransmitter linked to cognitive function, muscular contraction, and other neurobiological functions. AChE activity inhibition can be a therapeutic strategy. High throughput screening technologies require homogenous cell-based assays to effectively discover AChE inhibitors from sizable chemical libraries (Li et al., 2017)

In vitro AChE inhibition assays are crucial for detecting possible chemical dangers and ranking these compounds for additional toxicological testing because of the toxicological testing because of the toxicological consequences caused by AChE inhibition. Furthermore, the development of predictive toxicological models like quantitative structure-activity relationships may be facilitated by AChE inhibition experiments. AChE inhibition assays have been widely used in manual, lower-throughput formats but when dealing with large chemical libraries, their use in quantitative high-throughput screening platforms will enhance the capacity to identify chemical hazards and create predictive models (Li et al., 2022).

Numerous techniques, including colorimetric, spectrophotometric, fluorometric, and electrochemical methods have been developed to quantify the AChE activity. An accurate technique for assessing the AChE activity is Ellman’s

approach. The most widely used assay is based on Ellman's approach and uses 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) and acetylthiocholine as an alternate substrate. The shift of electrons to the sulfur atom causes the reaction to produce 5-thio-2-nitrobenzoate, which has a yellow hue (Hanumanthappa et al., 2024).

Table 1: ***Synthesis of the Study.***

AUTHOR/S	TITLE	YEAR	RESEARCH GAP
Vital, P.G.,Rivera, W.L.	Antimicrobial activity, cytotoxicity, and phytochemical screening of *Voacanga globosa* (Blanco) Merr. leaf extract (Apocynaceae)	2011	The study focuses on investigating the antimicrobial, antiprotozoal, cytotoxic, and phytochemical properties of *Voacanga globosa* (Blanco) Merr. leaf extract. Results showed that leaf extract has significant antibacterial effects against several bacteria (e.g., Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa) and antifungal activity against Candida albicans. The extract also exhibited antiprotozoal activity comparable to the standard drug metronidazole, inhibiting Trichomonas vaginalis and Entamoeba histolytica. Cytotoxic effects were observed, showing apoptotic-like changes in the protozoan parasites. However, the study uses in vitro methods thus a research gap exists in exploring the efficacy and safety of V. globosa extracts in in vivo models. The study also identified some bioactive compounds but did not isolate or fully characterize all the specific compounds responsible for the antimicrobial and antiprotozoal effects. Lastly, the study did not thoroughly explore the potential toxicity in human cells or the therapeutic window.
Macabeo, A.G., Vidar, W.S.,et.al.	Mycobacterium tuberculosis and cholinesterase inhibitors from Voacanga globosa	2011	The study identifies and describes new alkaloids, including globospiramine and deoxyvobtusine, that exhibit promising activities against mycobacteria and have potential anticholinesterase effects; however, the complete therapeutic potential and mechanisms of action of these compounds have not been thoroughly explored. Moreover, although the study suggests a new biogenetic pathway for developing the spiro- Aspidosperma- Aspidosperma framework, further research into the biosynthetic mechanisms and structural variety of related alkaloids in *Voacanga globosa* could broaden the range of prospective candidates.
Guzman, R.M.S., et.al.	Biphasic inflammatory effects of *Voacanga globosa* ethanolic leaf extract in ICR mice	2013	This study assessed the basic inflammatory effects of *V. globosa* in 6 to 8 weeks old female ICR mice. Immunomodulation could result in either potentiating the immune response or suppression of the reaction. The study has not further investigated how introducing a higher dose leads to suppressed immune response and focused solely on acute effects at specific doses. In addition, the study demonstrates that *V. globosa* has immunomodulatory effects, but it does not specify the active components that are responsible for this action.
Acebedo, A.R.,et.al	Apoptosis-Inducing Activity of HPLC Fraction from *Voacanga globosa* (Blanco) Merr. on the Human Colon	2014	This study aimed to scientifically validate the therapeutic properties of *Voacanga globosa* by evaluating the cytotoxic and pro-apoptotic effect of HPLC fractions from its leaves on HCT116 human

	Carcinoma Cell Line, HCT116		colon carcinoma and A549 human lung carcinoma cell lines. Results showed that the MP3 fraction causes apoptosis in HCT116 cells by reducing the mitochondrial membrane potential, which is essential for cell viability. Although the study identified the bioactive compounds of the plant, it did not fully isolate or characterized the compounds responsible for its cytotoxicity.
De Jesus, M.S.M.,et.al.	*Voacanga globosa* Spirobisindole Alkaloids Exert Antiviral Activity in HIV Latently Infected Cell Lines by Targeting the NF-κB Cascade: In Vitro and In Silico Investigations	2024	With an emphasis on the potential to reduce viral replication and infectivity, the study aimed to examine the inhibitory effects of globospiramine alkaloids from *Voacanga globosa* on HIV gene transcription. The study specifically looks at how globospiramine affects the NF-θB signaling pathway, which is implicated in HIV transcription, and how it affects TNF-α-induced viral replication in HIV-infected cell lines. The study focuses mainly on NF-κB as the key target for globospiramine and mentions Tat-mediated transcription inhibition for roscovitine. However, HIV replication is a multi-step process, involving enzymes like reverse transcriptase, integrase, and protease, as well as cellular factors. Investigating whether these compounds affect other stages of the HIV life cycle would provide a more comprehensive understanding of their potential as anti-HIV agents.
Manzano, J.A.H., et.al.	Globospiramine from *Voacanga globosa* Exerts Robust Cytotoxic and Antiproliferative Activities on Cancer Cells by Inducing Caspase-Dependent Apoptosis in A549 Cells and Inhibiting MAPK14 (p38α): In Vitro and Computational Investigations	2024	The study explored the anticancer mechanisms of Globospiramine, a spirobisindole alkaloid from *Voacanga globosa* through a combination of cell-based assays, computational analysis, and in silico simulation experiments. Results have shown that it exerts high cytotoxic activity against A549 lung cancer and MB-231 breast cancer cells, induced apoptosis by caspase activation and PARP-1 cleavage, disrupted mitochondrial dysfunction and target MAPK14 to inhibit cell proliferation and survival. However, the research highlights the need to validate the in silico studies of globospiramine as MAPK14 inhibitors of MB-231 cells as they have specific molecular characteristics which may necessitate in vitro and in vivo studies.
Manzano, J.A.H., et.al.	Cytotoxic, Antiproliferative and Pro-apoptotic Activities of Spirobisindole Alkaloids from the Philippine Medicinal Plant *Voacanga globosa*	2024	The study aims to investigate the cytotoxic and antiproliferative activities of spirobisindole alkaloids from *Voacanga globosa*. The results of the study showed that globospiramine may trigger the activation of caspase-3, indicating that the cancer cell underwent cell death. Further investigation on globospiramine is required to figure out its exact mechanism of action and to maximize its potential as a drug pharmacophore.
Macabeo, A.P.G et.al.	Mycobacterium tuberculosis and cholinesterase inhibitors from Voacanga globosa. *European Journal of Medicinal Chemistry*, *46*(7), 3118–3123. https://doi.org/10.1016/j.ejmech.2011.04.025	2011	Globospiramine demonstrated significant activity against *Mycobacterium tuberculosis* (H37Rv strain), and several of these compounds effectively inhibited the enzymes acetylcholinesterase and butyrylcholinesterase, particularly deoxyvobtusine. Globospiramine shows strong in vitro activity against tuberculosis while deoxyvobtusine demonstrates significant inhibition of butyrylcholinesterase (BChE). This study increases the diversity of alkaloid structures known to possess

			both antimycobacterial and anticholinesterase activities, specifically excluding flavonoids.
Olaleye, et.al	Antioxidant And Anti-Inflammatory Properties Of A Flavonoid Fraction From The Leaves Of *Voacanga Africana*	2005	Flavonoids in extracts from *Voacanga africana* leaves show potent anti-inflammatory and anti-nociceptive properties as evidenced by reduced carrageenan-induced edema and lower granulomatous tissue formation in the cotton pellet test. Additionally, the extracts reduced vascular permeability and attenuated both neurogenic and inflammatory pain phases in the formalin test, suggesting a dual mechanism involving neurogenic and inflammatory pathways. This aligns with traditional uses of *Voacanga africana* for pain relief and inflammation. However, a research gap exists in identifying the specific bioactive compounds responsible for these effects and understanding their mechanisms of action in relation to known inflammatory mediators.

Despite extensive research on the indole alkaloids of *Voacanga globosa* (e.g., deoxyvobtusine, globospiramine, vobtusine) for their antimicrobial, antiprotozoal, anti-tuberculosis, anticholinesterase, immunomodulatory, anticancer, and antiviral properties, the potential of its flavonoid compounds remains largely unexplored (Qin et al., 2022). This focus on indole alkaloids creates a significant research gap, potentially overlooking valuable bioactive compounds within the flavonoid fraction. Current literature on the antioxidant activity of the flavonoid fraction of Voacanga globosa illustrates this gap. Although Olaleye et al. (2005) had examined a flavonoid fraction from leaves of its close relative, Voacanga africana, an extensive investigation into Voacanga globosa's own flavonoid profile and bioactivities remains absent.

CHAPTER 3

Methods and Procedures

This chapter presents the methods and procedures involved in the pursuit of the study, which were adapted from various sources and was modified to fit the resources accessible to the researchers.

Research Methodology and Design

This research utilized a quantitative-experimental research design. Researchers performed phytochemical screening of the crude methanolic extract of Bayag-usa (Voacanga globosa). Flavonoids were isolated from the methanolic extract through solvent fractionation to yield the aqueous, ethyl acetate, and hexane fractions. The crude extract and the flavonoid-rich fraction of the plant extract were subjected to (1) Instrumental Analysis: Fourier Transform Infrared Spectroscopy (FTIR), Total Phenolics Content (TPC) Assay, Total Flavonoid Content (TFC) Assay; (2) Biochemical Assay; Cupric Reducing Antioxidant Capacity (CUPRAC) Assay and Acetylcholinesterase (AChE) Inhibition Assay.

Setting of the Study

This study was conducted at Centro Escolar University - Manila, University of the Philippines - Los Baños and University of the Philippines - Diliman. Crude extraction, solvent fractionation, confirmatory tests for the presence of flavonoids, and Fourier Transform Infrared Spectroscopy was conducted at CEU-Manila. Total Phenolic and Total Flavonoid Content, DPPH Inhibition Assay and CUPRAC Assay was conducted at BIOTECH UP-Los Banos- Food Laboratory 1. UP-Diliman Terrestrial and Natural Products Laboratory was selected due to the availability of the Acetylcholinesterase Inhibition Assay.

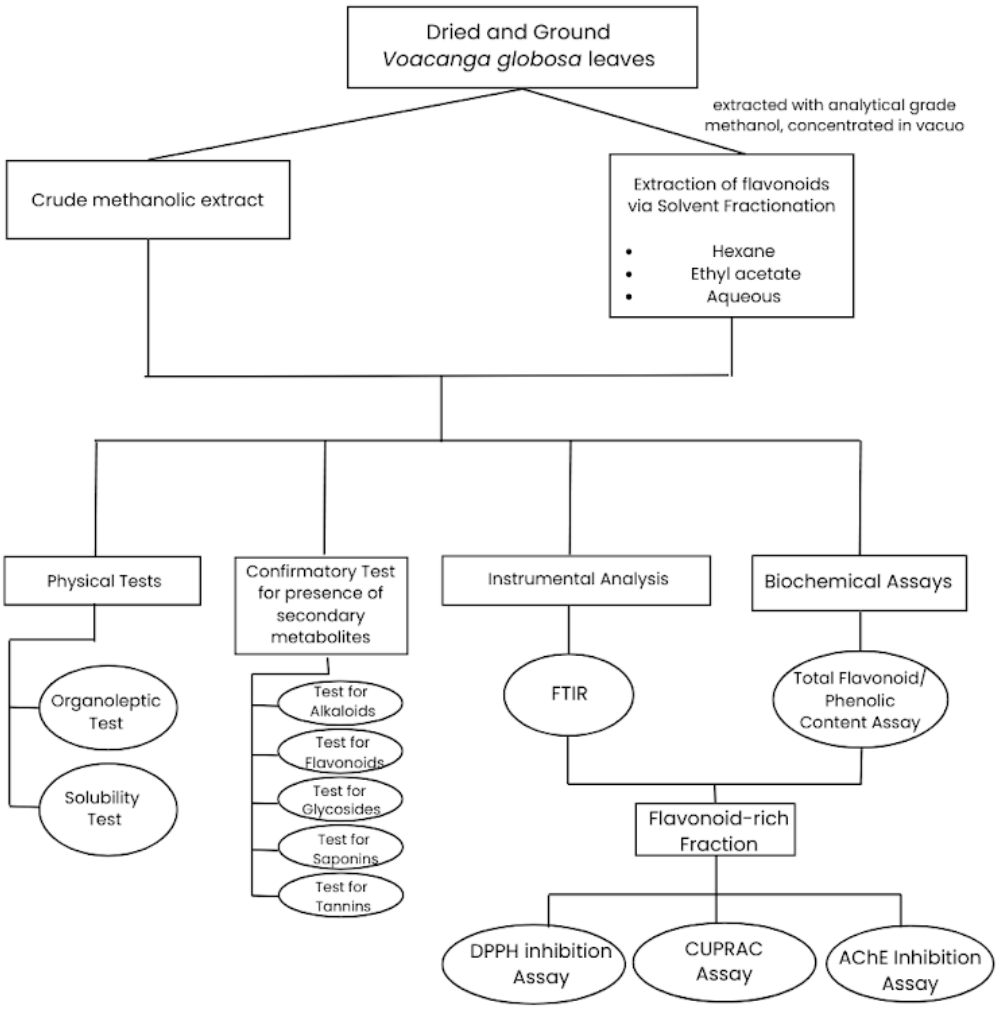

Figure 2: ***Schematic Diagram of Research Methodology.***

Research Procedure

Collection and Preparation of Plant Material

The leaves of *Voacanga globosa* (Bayag-usa) were collected at Alaminos, Laguna. The plant was identified and authenticated by the Bureau of Plant Industry located at Malate, Manila. Then, the leaves were shade-dried at room temperature for a five-day period.

The prepared leaves of the plant material were grinded into fine powder using a blender. The sample was weighed and placed in a 500 mL erlenmeyer flask. The powdered plant material was macerated with a sufficient amount of 80% methyl alcohol to completely submerge the material for 24-48 hours. Consequently, it was filtered and the residue was discarded. The filtrate was collected and concentrated using a rotary evaporator to separate the solvent from the extract. The extract was further concentrated to about 50 mL at a controlled temperature at 40°C until a syrupy mass was produced.

Formula for Percentage Yield

The concentration of the collected extracts in grams of dried plant material per mL extract was computed using this formula:

$$Percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ plant\ sample}\ x\ 100$$

Preparation of *Voacanga globosa* Extracts

A part of the methanolic extract was suspended in 200mL distilled water and was fractionated according to polarity.

The crude methanolic extract of *Voacanga globosa* that was dissolved in water and partitioned using n-hexane. The aqueous layer was drained, and the hexane layer was collected. The extract was subjected to complete defatting by repeating the extraction until the hexane layer was clear. The combined hexane layer was concentrated in vacuo, and weighed. Then, the aqueous layer was partitioned in the same manner with ethyl acetate to obtain the ethyl acetate fraction. The remaining aqueous layer was also evaporated to dryness to obtain the aqueous fraction. This was stored at 4°C for further analysis. The weight and percentage yield of all fractions were also determined.

Physical Test

Organoleptic Test

The color, odor, and physical state of the crude extract and its fractions of the extract was determined.

Solubility Test

The solubility of the crude extract and its fractions was tested by adding 1mL each of distilled water, 80% alcohol, chloroform, hexane, and ether on separate test tubes containing 1g of the samples. The test tubes were shaken vigorously and their solubility with the solvents was assessed by the development of a heterogeneous solution, color change, or release of gas or heat (Poquiz et.al., 2017). (Poquiz et.al., 2017).

Confirmatory Test for the Presence of Secondary Metabolites

Phytochemical screening was also performed as a test for confirmation of the presence of relevant phytochemicals. During this research, the researchers tested the methanolic extract and fractions for the presence of secondary metabolites e.g. alkaloids, flavonoids, tannins, glycosides, and saponins.

Test for Alkaloids

Wagner's Test

2 mg of the extract was treated with 1.5% v/v hydrochloric acid, followed by the addition of a few drops of Wagner's reagent. The formation of a yellow or brown precipitate indicated a positive result for alkaloids.

Mayer's Test

2 mg of the extract was treated with a few drops of Mayer's reagent. The appearance of white or pale yellow precipitate confirmed the presence of alkaloids.

Dragendorff's Test

2 mg of the extract was measured and mixed with 5 mL of distilled water and 2M hydrochloric acid until the mixture became acidic. Then, 1 mL of Dragendorff's reagent was added. The orange or orange-red precipitate formed, indicated the presence of alkaloids.

Test for Flavonoids

Shinoda Test

Ten drops of dilute hydrochloric acid and a small piece of magnesium ribbon were added to 0.5 mL of the plant extract. The presence of flavonoids was indicated by a pink, reddish, or brown color.

Lead Acetate Test

1 mL of crude methanolic extract was taken, and a few drops of 10% lead acetate solution were added. The presence of flavonoids was indicated by a yellow precipitate.

Alkaline Reagent Test

Two milliliters of crude methanolic extract were measured, and two to three drops of sodium hydroxide solution were added. Initially, a deep yellow color was observed, but it slowly turned colorless after the addition of a few drops of dilute HCl, signifying the presence of flavonoids.

Test for Saponin

Froth Test

A few milligrams of each extract were taken and mixed separately with 5 mL of distilled water. The mixtures were shaken well, and the formation of persistent foam indicated a positive result.

Test for Tannins

Ten grams of the plant extract were measured, ensuring an equivalent volume, and evaporated over a steam bath until nearly dry. The resulting residue was then dissolved in 20 mL of hot distilled water, followed by the addition of 5 drops of a 10% sodium chloride solution.

Ferric Chloride Test

Three drops of ferric chloride reagent were placed on one portion of the sample extract, and was done in the same manner with the tannic acid solution on another portion. A resulting mixture of brownish-green, indicated the presence of non-hydrolyzable (or condensed) tannins. A blue-black color, on the other hand, signified the presence of hydrolyzable tannins.

Test for Glycosides

Keller-Killiani Test

Three mg of each extract was measured and mixed with 3 mL of concentrated acetic acid. Then a single drop of 5% FeCl3 solution was then added, followed by a few drops of concentrated sulfuric acid. A reddish-brown ring formed at the interface between the layers to indicate a positive result.

Borntrager's Test

A small amount of each extract was taken and mixed with dilute HCl. The mixture was boiled for 5 minutes, cooled, and then shaken with an equal volume of chloroform, benzene, or another organic solvent. After separating the organic layer, it was treated with ammonia. A positive result was confirmed if the color of the aqueous alkaline layer turns pink to red.

Instrumental Analysis

Fourier Transform Infrared Spectroscopy (FTIR)

In this study, FTIR Analysis was performed to examine the flavonoid-rich fraction of *Voacanga globosa* using infrared light and detect their chemical characteristics. The semi-solid samples were placed directly on the ATR crystal, and pressure was applied using the clamp to ensure good contact. The sample spectrum was then collected using the Agilent Cary 360 software, and was scanned with a resolution of 4 cm-1 in the 400-4000 cm-1 range. This was done in two trials for each fraction.

The expected characteristic infrared absorption patterns of flavonoids is found between 4000 and 1300 cm-1.

Biochemical Assays

Total Flavonoids Content (TFC) Assay

To find the Total Flavonoid Content (TFC), one of the methods was to employ an aluminum chloride (AlCl3) solution. AlCl3 was found to react with the keto and/or hydroxyl moieties in flavonoid molecules to form acid-stable complexes that had a purple color. The purple color was quantified using a spectrophotometer at a wavelength of 570 nm. Total flavonoid content was subsequently calculated from a standard curve established with varying levels of Catechin, a flavonoid antioxidant. To derive this standard curve for the TFC assay, Catechin was analyzed at 10, 15, 20, 25, 30 µg/µl and the corresponding values of absorbance were plotted on an XY plot. The linear equation derived from this plot is:

$$y(absorbance) = m * x(concentration) + b$$

By applying this Catechin standard curve, the total flavonoid content of the sample was determined as Catechin equivalent (CE) in mg/g of sample by using Beer-Lambert Law of Absorbance:

$$TFC\ (CE\ mg/g) = C * DF * V/m$$

where;

- C = catechin concentration, mg/ml
- DF = dilution factor
- V = volume analysed in sample, ml
- m = mass tested in sample, g

TFC test samples were assayed in triplicate at 10, 15, 20, 25, and 30 µg/µl concentrations.

Total Phenolic Content Assay (TPC)

The reagent used in the Total Phenolics Content (TPC) Assay was the Folin-Ciocalteu (F-C) reagent, a yellow-colored liquid reagent made up of phosphotungstic and phosphomolybdic acid compounds. Phenols were reduced to a dark blue product by being in an alkaline environment. The colorimetric reaction was quantified using a spectrophotometer at 760

nm. The obtained absorbance values were used in the calculation of the total phenolic content through comparison of a standard plot made using various concentrations of Gallic Acid, a well-known antioxidant phenolic compound. Gallic Acid was tested for concentrations 10, 15, 20, 25, 30 µg/µl for preparing a standard curve of the TPC assay. The obtained absorbance values were graphed on an XY plane, and the linear equation was calculated as:

$$y(absorbance) = m * x(concentration) + b$$

The total phenolic content of the sample was interpolated and calculated as gallic acid equivalent (GAE) mg/g sample, using the Gallic Acid standard curve and a formula grounded in the Beer-Lambert Law of Absorbance:

$$TPC\ (GAE\ mg/g) = C * DF * V/m$$

where;

- C = concentration (gallic acid), mg/ml
- DF = dilution factor
- V = volume of sample tested, ml
- m = mass of sample tested, g

For the TPC assay, samples were analyzed at concentrations of 10, 15, 20, 25, 30 µg/100µl in three (3) trials.

DPPH Free Radical Scavenging Assay

To measure DPPH radical scavenging activity, a reaction mixture was prepared by adding 100 μL of the flavonoid-rich fraction at the different concentrations, specifically 10, 15, 20, 25, and 30 $\mu g/\ \mu L$ or methanol (for the control) to 5 mL of a 0.1 mM DPPH (Sigma-Aldrich D9132) solution methanol. The mixture was left to stand for 20 minutes to allow the reaction to occur. After that, the absorbance was measured using a double-beam UV-Vis spectrophotometer (Shimadzu UV1601) at 517 nm, with distilled water used to zero the instrument. Each sample was tested in triplicate, and the percentage of inhibition was calculated using the formula below (Rebeiro et.al., 2008).

$$\%\ Inhibition = \left[\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}\right] \times 100$$

Cupric Reducing Antioxidant Capacity (CUPRAC) Assay

The antioxidant capacity of Voacanga globosa flavonoid fraction was also investigated through a modified CUPRAC assay method by BIOTECH UPLB Food Laboratory (Alpinar et al., 2009). This involved the mixing of 1mL of 0.01M copper chloride, 1mL of 1.0M ammonium acetate, and 1mL of 0.75mM neocuproine to obtain a fresh working solution.

The solutions were in the concentrations of 10, 15, 20, 25, and 30 g/L, and the triplicate experiments were performed against Ascorbic Acid as reference drug. Voacanga globosa leaf extract and fractions were treated with this solution for 15 minutes. Distilled water, on addition, was analyzed after the produced product for absorbance at 450 nm to observe the reducing power of the flavonoid fraction. In the present study, authors have compared the TPC of fractions and crude extract.

Acetylcholinesterase (AChE) Inhibition Assay

Acetylcholinesterase (AChE) Inhibition Assay was employed to ascertain the ability of the flavonoid fraction of Voacanga globosa to inhibit acetylcholinesterase (AChE). This was assessed using spectrophotometric methods according to Ellman's method with slight modifications (Acero & Amor, 2022).

The inhibitory activity of AChE was determined by spectrophotometry at 25°C and pH 8.0. 80 µL reaction mixture of buffered Ellman's reagent containing 10.0 mM Dinitrobenzoic acid and 17.9 mM Sodium bicarbonate, pH 7.0, 10 µL of 1.0 U/mL Acetylcholinesterase enzyme, 180 µL of 100 mM pH 8.0 phosphate buffer, and 15 µL of methanol solution of Voacanga globosa fraction were added to a 96-well plate. *Voacanga globosa* fractions were also dissolved in small amounts of DMSO as a co-solvent if there are solubility limitations with methanol and were diluted to a concentration range of 100, 150, 200, 250, 300, 350, 400, 450, 500, 1000 ppm. This was incubated at 25 °C for a period of 15 minutes, after which the reaction was activated by introducing 15 µL of 94 µM acetylthiocholine iodide substrate to the reaction mixture. The absorbance was spectrophotometrically detected at 420 nm using a microplate reader. For a duration of 10 minutes, the reaction was continuously monitored at 20 second intervals. This process was done in duplicate. The following formula was used to calculate the percent inhibition.

$$\%\ Inhibition = \left[\frac{Absorbance_{(-)\,control} - Absorbance_{sample}}{Absorbance_{(-)\,control}}\right] \times 100$$

A negative control, without the sample extract, was tested under the parallel set of concentrations and conditions. A positive control, Galantamine was used.

Computation of IC50

The IC50 or half-maximal inhibitory concentration was determined through evaluation of the percentage inhibition of the plant sample across the given range of concentrations and fitting the data to a regression model. For each concentration, the percentage inhibition was calculated and consequently plotted against the logarithm of concentration, and a dose-response curve is generated. The IC50 value is determined from the equation of the curve as the concentration at which 50% inhibition occurs. This was performed using GraphPad Prism.

Statistical Treatment

To assess and compare the means of the different concentrations of the Voacanga globosa ethyl acetate fraction and the positive controls, independent t-test, and one-way ANOVA followed by post hoc analysis (Tukey's HSD Test) were used.

Descriptive statistics were used to summarize the dataset, while the Shapiro-Wilk test assessed the normality of data distribution. Additionally, Levene's test was conducted to evaluate the homogeneity of variances across groups, guiding the selection of appropriate analytical methods.

For comparisons of two independent groups, an independent t-test was applied when data were normally distributed.

For the situation with greater than two groups, a one-way analysis of variance (ANOVA) was used for parametric data.

If differences were found to be significant, post-hoc pairwise comparisons were conducted using Tukey's Honest Significant Difference (HSD) test. Results are presented as mean ± standard deviation where p = 0.05 was taken as significant.

CHAPTER 4

Presentation, Analysis, and Interpretation of Data

This chapter reports the outcomes of experiments conducted to study the properties of Voacanga globosa extract and fractions. This chapter contains a description of the physical appearance of the samples of the plant, the percentage yield of some of the extracts, as well as the results of phytochemical screenings. Instrumental determinations like FTIR and biochemical assays for total phenolic content, total flavonoid content, antioxidant activity, and acetylcholinesterase inhibition of the flavonoid fraction are also given.

1. Physical Description of the Samples and Percentage Yield

The dried crude methanolic extract and hexane, ethyl acetate, and aqueous fractions all yielded a brownish green semisolid thick syrupy mass. All the extracts had a tea-like odor and were semisolid at room temperature.

Table 2: ***Percentage Yield of Voacanga globosa Crude Methanolic Extract (VGM), Hexane (VGH), Ethyl Acetate (VGE), and Aqueous (VGA) Fractions.***

Extract	Pulverized Weight	Weight After Drying	Percentage Yield
VGM	900g	74.4 g	8.22%
VGH		0.70 g	0.07%
VGE		6.6 g	0.73%
VGA		13.0g	1.44%

Table 2 also indicates that the extraction yield of Voacanga globosa with different solvents differ significantly. The highest extraction of 8.22% was obtained using the crude methanolic extract (VGM) and illustrated that methanol was the best solvent to use in extracting a variety of bioactive compounds from the plant. This is then followed by aqueous fraction (VGA) having a yield of 1.44%, ethyl acetate fraction (VGE) with a yield of 0.73%, and lastly by the hexane fraction with a yield of 0.07%.

Table 3: ***Solubility of Voacanga globosa Crude Methanolic Extract (VGM), Hexane (VGH), Ethyl Acetate (VGE), and Aqueous (VGA) Fractions.***

SOLUBILITY TEST					
Extract	**Distilled Water**	**N-hexane**	**Chloroform**	**Alcohol**	**Ether**
VGM	Soluble	Insoluble	Insoluble	Soluble	Insoluble
VGE	Insoluble	Insoluble	Insoluble	Soluble	Insoluble
VGH	Insoluble	Soluble	Soluble	Insoluble	Insoluble
VGA	Soluble	Insoluble	Insoluble	Soluble	Insoluble

Table 3 describes the solubility pattern of Voacanga globosa methanolic crude extract and fractions to illustrate the polarity-based nature of compound extraction. Alcohol and distilled water solubility of the crude extract revealed the presence of polar as well as moderately polar nature of compounds. Ethyl acetate fraction alcohol and n-hexane solubility confirmed there are intermediate polarity compounds that can be dissolved in both nonpolar and polar solvents. Similarly, hexane fraction solubility in hexane and chloroform confirmed there are more nonpolar compounds that are predominant that favor lipophilic solvents. Lastly, the solubility of the aqueous fraction in distilled water and

alcohol indicated higher concentrations of polar compounds which easily dissolve in alcohol or water. The trend in solubility supports the effectiveness of the fractionation process in separation by polarity.

2. Confirmatory Test for the Presence of Secondary Metabolites

Table 4: ***Results of Confirmatory Test for Secondary Metabolites of Voacanga globosa Crude Methanolic Extract (VGM), Hexane (VGH), Ethyl Acetate (VGE), and Aqueous (VGA) Fractions.***

Test	VGM	VGH	VGE	VGA	Secondary metabolite detected
Wagner's Test	+	-	-	-	Alkaloids
Mayer's Test	+	-	-	-	
Dragendroff's Test	+	-	-	-	
Keller Killiani Test	-	+	+	+	Cardiac Glycosides
Borntrager Test	-	-	-	-	
Froth Test	+	+	-	+	Saponin
Alkaline Reagent Test	+	-	+	-	Flavonoids
Shinoda Test	-	-	+	-	
Lead Acetate	+	+	+	+	
FeCl Test	+	-	-	+	Tannins (nonhydrolyzable)

(+) Positive results (-) Negative results

The phytochemical constituents identified in the crude methanolic extract and its fractions are listed in Table 4. The crude methanolic extract gave positive tests for alkaloids, saponins, cardiac glycosides, flavonoids, and non-hydrolyzable tannins. The hexane fraction tested positive for saponins and glycosides, while the ethyl acetate fraction tested positive for glycosides and flavonoids. The aqueous fraction tested positive for glycosides, saponins, flavonoids, and non-hydrolyzable tannins.

This again reinforces the polarity-based fractionation, with hexane fraction consisting of nonpolar compounds, the ethyl acetate fraction of moderately polar compounds, and the aqueous fraction comprising highly polar compounds. It shows that flavonoids co-exist in aqueous as well as ethyl acetate fractions, further confirming their biphase solubility-based glycosylation, whereas methanolic extract has the whole suite of phytochemicals because of its versatile solvent nature (Nawaz et. al., 2020).

3. Instrumental Analysis

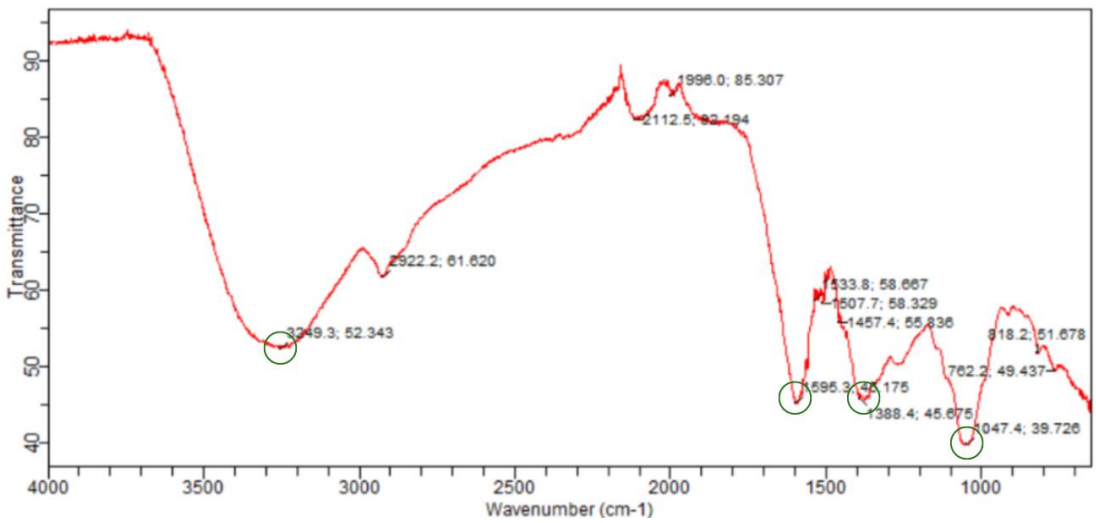

Figure 3: ***FTIR Results of the Crude Methanolic Extract of Voacanga globosa.***

The broad stretching at 3249.3 cm-1 is indicative of OH stretching vibrations found in the phenol and hydroxyl groups of flavonoids. The bending found at 1595.3 cm-1 showed the presence of aromatic rings while those found at 1388.4 and 1047.4 cm-1 is potentially correlated with C-H and C-O stretching respectively. These groups can be found in alcohols and ethers present in flavonoid compounds.

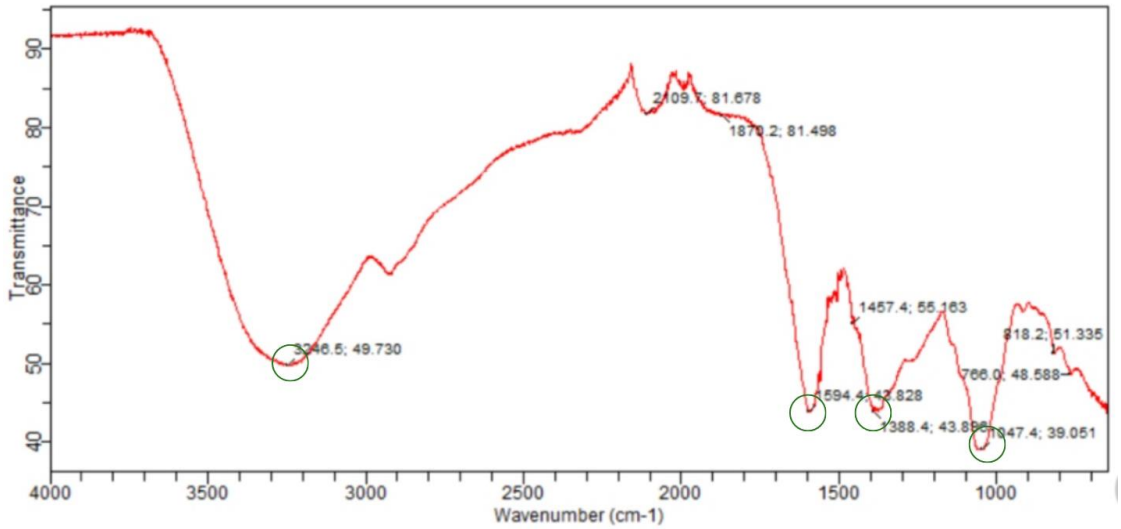

Figure 4: ***FTIR Results of the Aqueous Fraction of Voacanga globosa.***

The broad stretching at 3246.5 cm-1 showed characteristic alcohol and phenol groups generally present in flavonoids. This is followed by bending at 1594.4, 1388.4, and 1047.4 cm-1 which represent aromatic C-C, C-H, and C-O functional groups which are typically found in flavonoid glycosides.

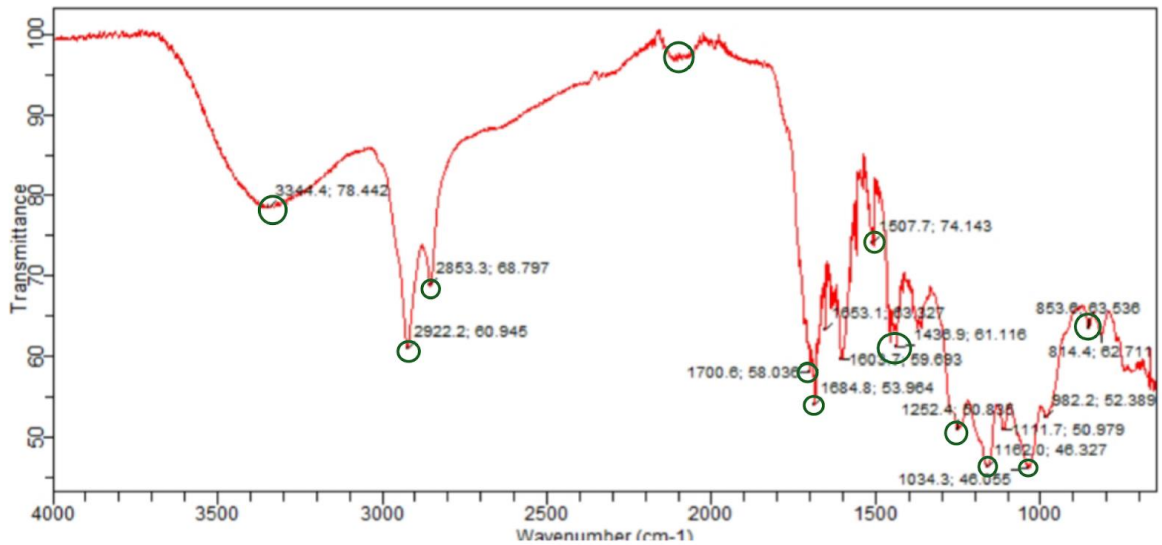

Figure 5: ***FTIR Results of the Ethyl Acetate Fraction of Voacanga globosa.***

The FTIR spectrum of the ethyl acetate fraction of *Voacanga globosa* exhibited characteristic absorption bands indicative of several key functional groups present in flavonoids. A broad stretching at 3344.4 cm^{-1} suggested the presence of hydrogen-bonded hydroxyl groups, consistent with alcohols and phenols. Narrow stretching at medium absorption bands, 2922.2 and 2853.3 cm-1, indicated the presence of C-H bonds representing aliphatic side chains such

as methyl and alkyl groups. Sharp intense peaks at 1700.6 and 1684.8 cm^{-1} pointed to carbonyl (C=O) functionalities, suggestive of the presence of flavonoid compounds. Furthermore, a peak at 1603.0 and 1436.9 cm^{-1} implied C=C-C aromatic ring stretching, potentially associated with flavonols and anthocyanins. Finally, absorption bands at the fingerprint region, 1252.4, 1162.0, and 1034.3 cm^{-1} indicated C-O vibrations, suggesting the presence of ethers and phenolic compounds within the fraction.

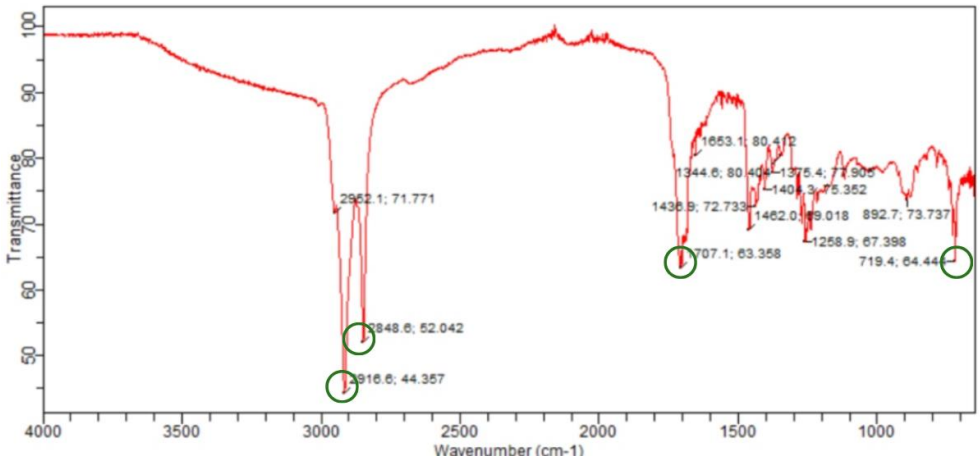

Figure 6: ***FTIR Results of the Hexane Fraction of Voacanga globosa.***

A strong narrow band between 2916.6 and 2848.6 indicated the presence of long -chain linear aliphatic compounds. These are indicative of the presence of non-polar hydrocarbons, fatty acids, waxes, and lipid-like substances in the hexane fraction. This is followed by medium peaks found at 1707.1 and 719.4 cm-1.

The FTIR instrumental analysis presents that compared to other fractions, the ethyl acetate extract displayed the most characteristic spectral features of flavonoids and phenols, supporting its selection as the most enriched fraction for these specific bioactive compounds.

4. Total Phenolic Content and Total Flavonoid Content

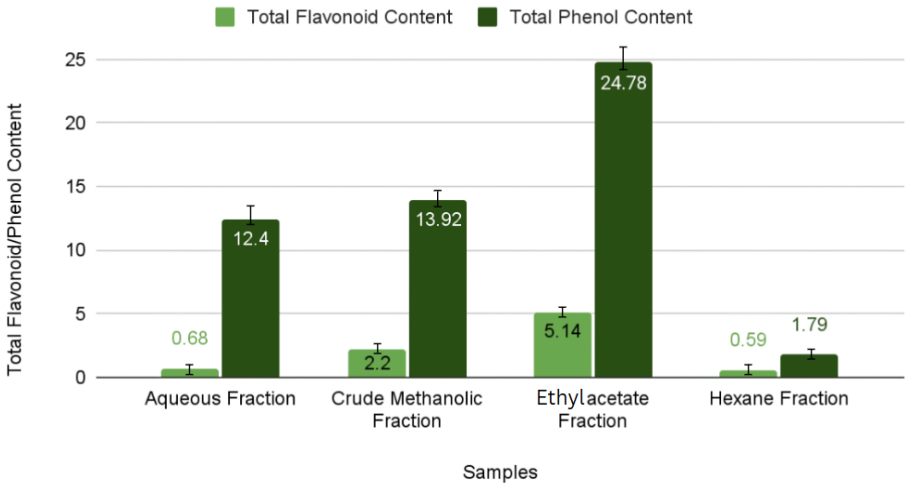

Figure 7: ***Total Phenolic and Flavonoid Content of Crude Methanolic Extract, Hexane Fraction, Ethyl Acetate Fraction, and Aqueous Fraction of Voacanga globosa.***

The Total Phenolic and Flavonoid content assays were utilized to select the flavonoid-rich fraction which was consequently analyzed for its antioxidant and acetylcholinesterase inhibitory activity.

Figure 7 shows that the ethyl acetate fraction exhibited a higher extraction of Phenolic and Flavonoid compounds compared to other fractions. The ethyl acetate fraction exhibited a total phenolic content at 24.78 ± 1.39 mg GAE/mL, followed by the crude methanolic extract at 13.92 ± 0.97 mg GAE/mL, aqueous fraction at 12.40 ± 0.80 mg GAE/mL and hexane fraction at 1.79 ± 0.04 mg GAE/mL. Similarly, the Total Flavonoid Content of ethyl acetate fraction is 5.14 ± 0.39 mg CE/mL, with lower amounts in the crude methanolic extract with 2.20 ± 0.04 mg CE/mL, aqueous fraction with 0.68 ± 0.06 mg CE/mL, and hexane fraction with 0.59 ± 0.03 mg CE/mL. The results show that the ethyl acetate fraction of Voacanga globosa has the highest TPC and also the highest TFC, consistent with the FTIR analysis which showed characteristic spectra of these specific bioactive compounds in this fraction.

These results also align with the solubility characteristics of flavonoids in ethyl acetate. According to Miao et.al. (2022), free flavonoids are generally soluble in organic solvents such as methanol, ethanol, ethyl acetate, chloroform, and ether, as well as in dilute alkaline solutions, but they exhibit a little to no solubility in water. In contrast, the presence of a sugar moiety enhances the solubility of flavonoids in water. Consequently, flavonoid glycosides are typically soluble in highly polar solvents like water, methanol, and ethanol, while they remain insoluble in nonpolar organic solvents such as benzene, chloroform, hexane, and ether.

However, the total phenolic and flavonoid content in the ethyl acetate fraction of this study was relatively lower compared to previous studies of plants within the same family as Voacanga globosa. Several factors may have contributed to this, including physical damage, environmental conditions, and specific hormones, all of which can influence the gene expression involved in flavonoid biosynthesis in plants. Additionally, the extraction method itself may have played a role in the reduced flavonoid yield (Valentin et al., 2024).

5. Antioxidant Assays

5.1. DPPH Free Radical Scavenging Assay

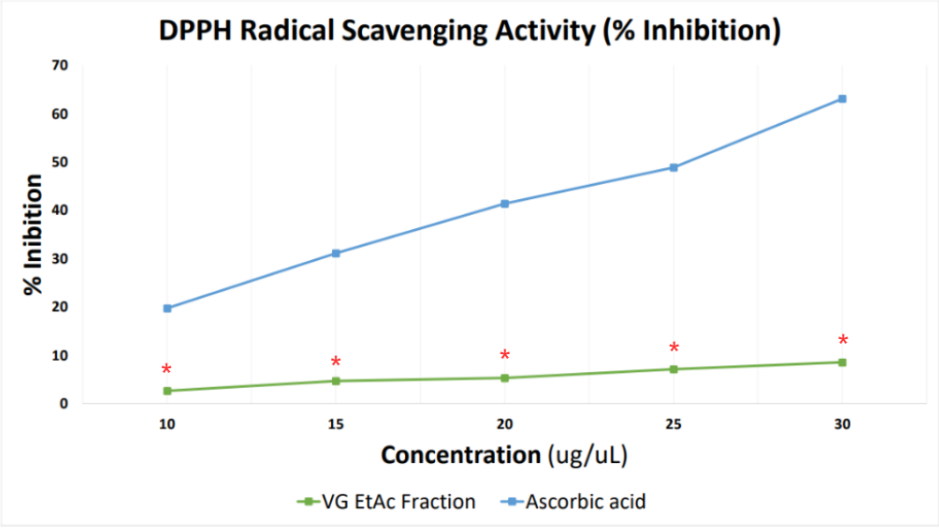

Figure 8: ***DPPH free radical scavenging activity of VGE and ascorbic acid.***

**vs ascorbic acid (positive control), p<0.05 is considered statistically significant (independent t-test)*

Figure 8 shows that *Voacanga globosa* ethyl acetate fraction has a relatively weak ability to neutralize free radicals as compared with the positive control, Ascorbic acid. Independent t-test showed significantly lower DPPH antioxidant activity, as it exhibited significant difference ($p < 0.05$) against the positive control. This result shows that none are comparable to the % inhibition of Ascorbic acid.

Among the tested concentrations, it showed the lowest activity at 10 ug/uL with 2.61 ± 0.20% inhibition, 4.64 ± 0.24% at 15ug/uL, 5.31 ± 0.09% at 20ug/uL, 7.09 ± 0.32% at 25ug/uL and the highest activity at 30 ug/uL which is 8.53 ± 0.26%. These results indicate that further increases in concentration may potentially enhance the activity significantly or other factors such as the solubility, concentration of bioactive compounds, binding affinity, and the lack of synergistic effects of flavonoids with other constituents of *Voacanga globosa* may have limited its activity.

This is consistent with the result of the Total Phenolic/ Flavonoid Content assay, where the relatively low concentration of these bioactive compounds may not be sufficient to exert a strong antioxidant effect, leading to the observed weak inhibition of free radicals. Therefore, increasing the concentration of these compounds could potentially enhance its ability to neutralize free radicals and improve its overall antioxidant potential. These findings also align with the observed positive correlation between total flavonoid content and antioxidant activity of the plant sample.

Phenolic compounds, specifically flavonoids, serve various biological functions including protection against oxidative damage. Moreover, flavonoids act as stabilizing agents for scavenging molecules that would otherwise be removed due to excessive ROS accumulation. This dual function is attributed to the high number of free hydroxyl groups which enhances the reactivity of flavonoid hydroxyl groups with oxidants (Mehmood et.al., 2022).

Table 5: ***IC50 of the DPPH Free Radical Scavenging Activity of VGE*.**

Sample	IC50 (ug/uL)
VGE	80.10 ± 9.14
Ascorbic acid	80.45 ± 5.85

**vs ascorbic acid (positive control), p<0.05 is considered statistically significant (Independent t-test)*

Table 5 shows the computed IC_{50} for DPPH inhibition of *Voacanga globosa* (80.10 ± 9.14 µg/µL) which has no significant difference ($p>0.05$) to that of ascorbic acid (80.45 ± 5.85 µg/µL). This suggests that *Voacanga globosa* may exhibit an antioxidant potency comparable to the standard, indicating its potential effectiveness in scavenging 50% of free radicals at approximately 80 µg/µL. These findings highlight its possible use as a natural antioxidant source, warranting further investigation into its mechanism and applications.

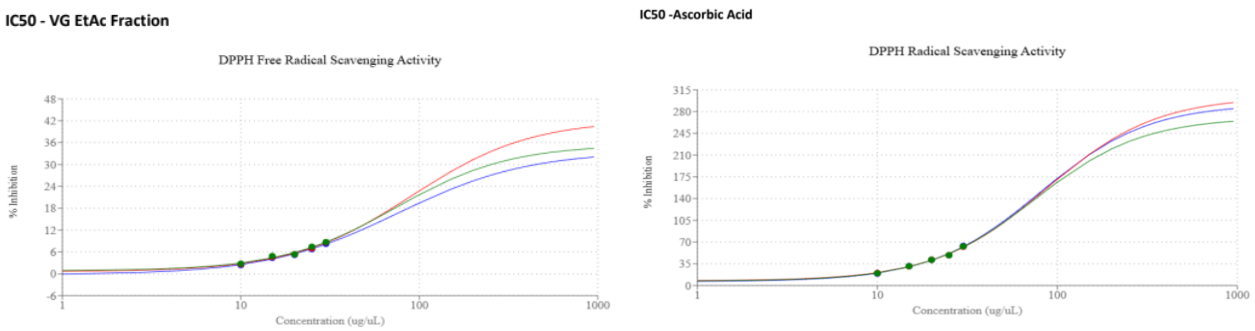

Figure 9: ***Dose Response Curve of the DPPH Inhibition of VGE vs Ascorbic Acid*.**

Figure 9 shows the DPPH Free Radical Scavenging Activity, showing a dose-dependent relationship between concentration (µg/µL) and % inhibition. The x-axis is on a logarithmic scale (1–1000 µg/µL), while the y-axis represents the percentage of free radical scavenging activity. Data points indicate an increasing trend, with fitted curves following a sigmoidal pattern. Initially, inhibition is low at lower concentrations, then rises with increasing concentration. However, the slope did not reach a plateau because of the lack of higher concentration. This trend suggests that the tested samples may possess antioxidant properties, although minimal, with higher concentrations potentially leading to greater free radical scavenging activity.

5.2. Cupric reducing antioxidant capacity CUPRAC Assay

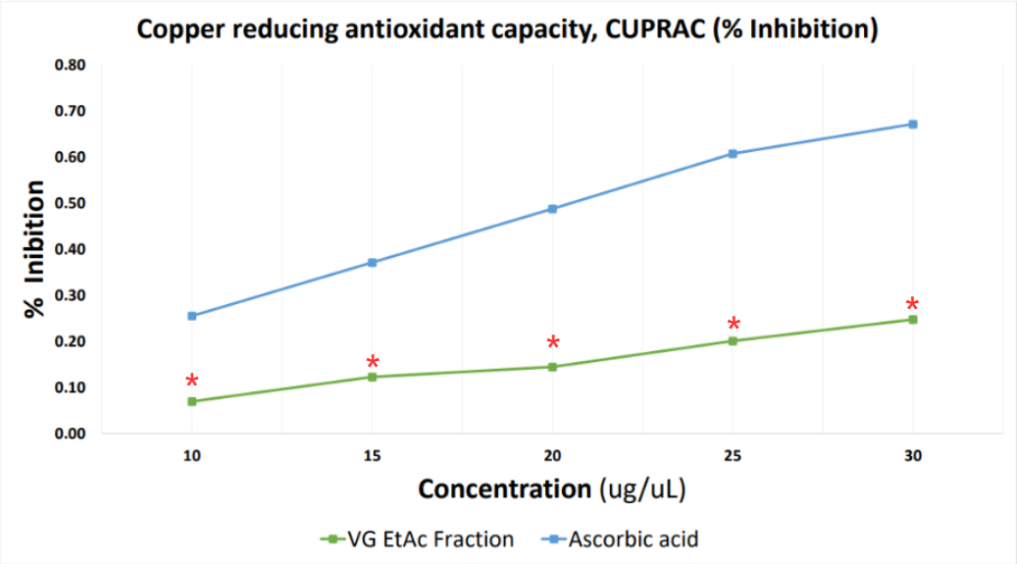

Figure 10: ***Cupric reducing antioxidant capacity of the ethyl acetate fraction of VGE vs Ascorbic Acid.***

**vs ascorbic acid (positive control), p <0.05 is considered statistically significant (independent t-test)*

Independent t-test shows significant difference (p <0.05) between the plant sample and the positive control in the cupric reducing antioxidant capacity (CUPRAC) assay suggests variability in their electron-donating abilities.The *Voacanga globosa* ethyl acetate fraction showed a relatively weak Cupric reducing antioxidant capacity against the standard, Ascorbic Acid. The VG EtAc fraction exhibited an inhibition of 0.07 ± 0.00% at 10 ug/uL, 0.12 ± 0.01% at 15 ug/uL, 0.14 ± 0.00% at 20g/uL, 0.20 ±0.00% at 25 ug/uL, and increasing gradually to 0.25 ±0.00% at 30 ug/uL. The ethyl acetate fraction of *V. globosa* tested in five different concentrations exhibited moderate reducing power, only reaching approximately 30% of the inhibition exhibited by the positive control, Ascorbic acid. Although this suggests that the inhibitory activity is not comparable with the standard drug, the dose-dependent increase further indicates that a higher concentration is necessary to attain similar activity.

Similarly, in a study of Ozakieoniso James Kemelayefa et al., (2024), *Parsonsia straminea*, from Apocynacea family, reported a Cupric reducing activity of 0.8± 0.00% at 31.3 ug/uL, which was significantly lower than the standard ascorbic acid, 9.28 ± 2.93. However, results also showed that at 1000 ug/uL, its reducing capacity is 49.5 ± 0.5%, implying a dose-related relationship. This trend aligns with the current data, suggesting that while *Voacanga globos*a exhibits weak reducing activity at lower concentrations, there is potential for increased efficacy at higher doses, similar to the pattern observed in *Parsonia straminea*. Furthermore, this suggests that optimizing concentration or identifying

specific bioactive compounds may enhance its antioxidant and inhibitory potential, making it a promising candidate for further investigation in neuroprotective and therapeutic applications.

Table 6: ***IC50 of the Cupric reducing antioxidant capacity of VGE*.**

GROUP	IC50 (ug/uL)
VGE	59.91 ± 3.30 *
Ascorbic acid	20.72 ± 0.42

**vs ascorbic acid (positive control), p<0.05 is considered statistically significant (Independent t-test)*

Table 6 shows that the VGE fraction possesses a significant difference with ascorbic acid in terms of the cupric reducing antioxidant capacity. The potency of a sample decreases with increasing computed IC50, and the potency increases with decreasing IC50 values, compared to the standard. This suggests that the ethyl acetate fraction of Voacanga globosa is less potent than ascorbic acid, with IC50 values of 59.91 ± 3.30 and 20.72 ± 0.42, respectively.

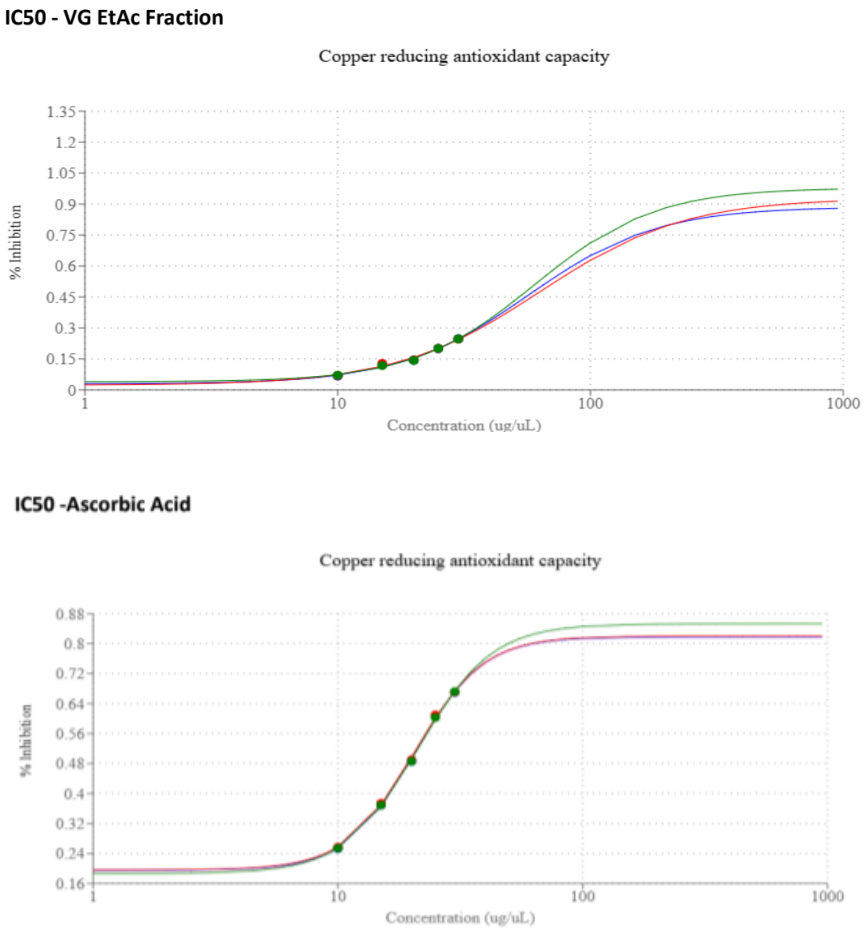

Figure 11: ***Dose Response Curve of the Cupric reducing antioxidant capacity of VGE vs Ascorbic Acid*.**

The data points follow a sigmoidal curve, indicating a dose-dependent response. This trend aligns with the computed IC50 values, suggesting that *Voacanga globosa* requires a higher concentration to achieve significant Cupric reducing antioxidant capacity compared to ascorbic acid. Ascorbic acid exhibits a steeper curve, reaching a higher % inhibition

at lower concentrations, highlighting its stronger antioxidant activity. In contrast, the VGE fraction shows a more gradual increase in inhibition, reinforcing the need for a greater concentration to achieve similar effects.

6. Acetylcholinesterase Inhibition Assay

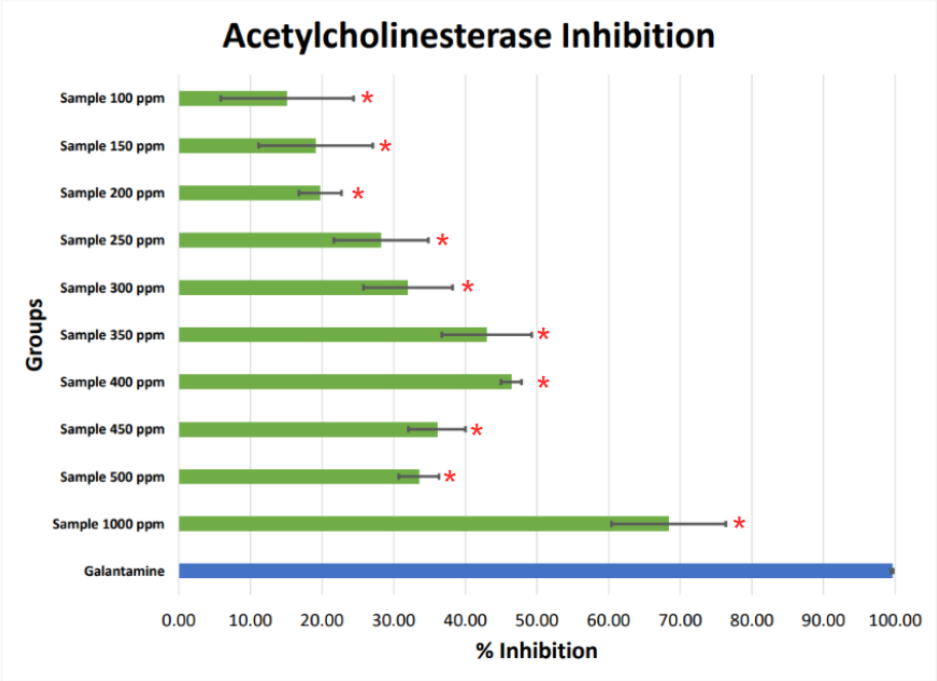

Figure 12: ***Acetylcholinesterase inhibition of VGE vs Galantamine.***

**vs Galantamine (positive control), p<0.05 (ANOVA with Tukey's HSD Test.)*

Figure 12 shows the acetylcholinesterase (AChE) inhibition activity of the ethyl acetate fraction of *Voacanga globosa* compared to Galantamine across various concentrations, ranging from 100ppm to 1000ppm. The ethyl acetate fraction of Voacanga globosa demonstrated a lower inhibition activity than the standard. However, it exhibited a gradual, dose-dependent increase from approximately 15.12% at 100 ppm to 68.39% at 1000 ppm. Fluctuations observed at around 400 to 450 ppm may indicate enzyme saturation at mid-range concentrations. In a study by Radic et.al. (1991), at high concentrations, substrates like acetylcholine can bind to the peripheral anionic site (PAS) of AChE, competing with compounds like propidium and causing substrate inhibition. This occurs when too much substrate binds to the PAS instead of just the active site, which interferes with the function of the enzyme.

ANOVA showed a significantly lower inhibitory activity than Galantamine, as it exhibited a statistically significant difference ($p < 0.05$) against the positive control. This result shows that none are comparable to the % inhibition of Galantamine. However, among the 10 analyzed concentrations, a significant inhibitory effect against acetylcholinesterase was observed at 1000 ppm, indicating that higher concentrations may be necessary to enhance its biological activity. Moreover, according to Gubler et.al. (2012), a sample is deemed active if it exhibits at least 50% inhibition and demonstrates a statistically significant difference from the negative control, with $p < 0.05$.

This trend aligns with findings reported by Macabeo et al. (2011), who identified that although *V. globosa* exhibits promising inhibitory activity in cholinesterase (AChE 87% at $10^{-4.3}\ M$, BChE IC_{50} 6.2), particularly against

butyrylcholinesterase, it also has lower activity compared to the standard drug, Galantamine. The results suggest that the extract has potential inhibitory activity, but not as potent as the pharmaceutical standard.

Moreover, studies indicate that *H. lancifolius* (uleine) belonging to the Apocynaceae family have also exhibited statistically significant acetylcholinesterase (AChE) inhibition, with p-values below 0.05. Among the crude extract fractions, the ethyl acetate fraction of *H. lancifolius* exhibited the highest inhibition (74.20 ± 2.3%) at 5 mg/mL indicating strong AChE inhibitory potential. Additionally, the purified uleine from *H. lancifolius* demonstrated a dose-dependent inhibition, ranging from low inhibition at 0.015 mg/mL to high inhibition at 1 mg/mL. Notably, at 1 mg/mL, purified uleine exhibited a highly significant inhibition ($p < 0.01$ or $p < 0.001$), reaching 88.93 ± 0.1%, compared to the standard physostigmine (1 mg/mL), which exhibited 98.82 ± 0.3% inhibition. This confirms the strong AChE inhibitory potential of uleine. (Seidl et al., 2010).

The results from Voacanga globosa in the current study can be viewed in the context of H. lancifolius's higher AChE inhibition, suggesting that further investigation into the active compounds in *Voacanga globosa* could lead to a better understanding of its acetylcholinesterase inhibitory potential. This could potentially guide future investigations aimed at purifying and enhancing the bioactivity of its extracts, similar to how purified uleine in *H. lancifolius* was shown to exhibit stronger inhibition.

Table 7: ***IC50 for Acetylcholinesterase Inhibition of Voacanga globosa Ethyl Acetate Fraction.***

GROUP	IC50
VGE	3817.1257 ppm
Galantamine (Girgin et.al, 2023)	556.01 uM or 159.71 ppm
Equation	$Y = -4.23 + \frac{251.2132 + 4.23}{1 + (\frac{x}{3817.1257})^{-0.8515}}$

Table 8 shows the IC_{50} value of VGE fraction which 50% inhibition of acetylcholinesterase (AChE) activity occurs. In this study, the equation above was used to derive the IC_{50} value of VGE which is 3817.13 ppm. This indicates that a high concentration is required to achieve half-maximal enzyme inhibition.

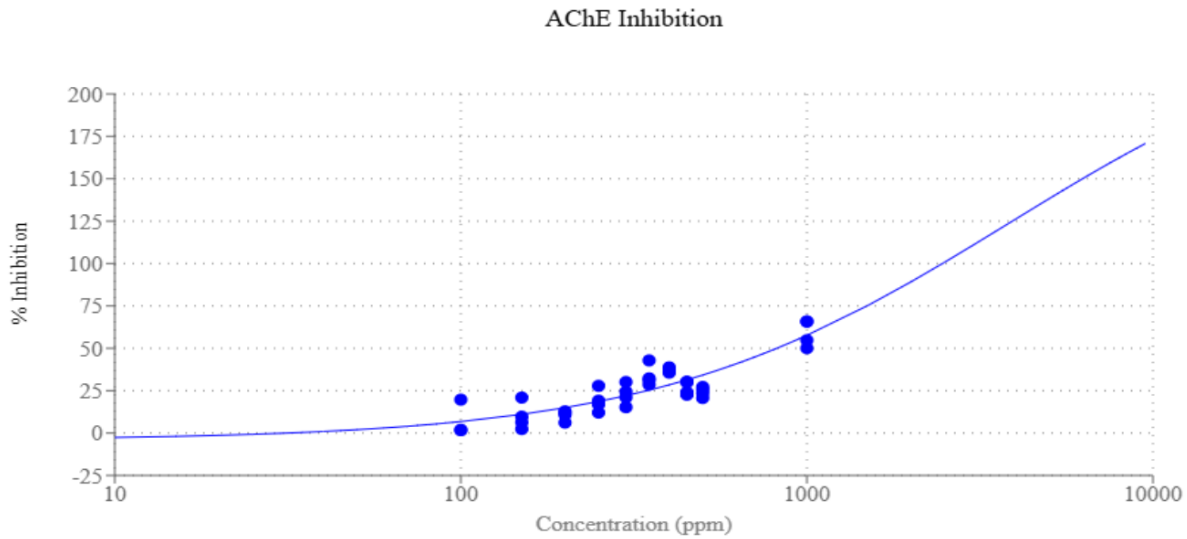

Figure 13: ***Dose Response Curve of the Acetylcholinesterase Inhibition of VGE Fraction.***

Figure 13 shows the relationship between concentration (ppm) and AChE inhibition, with the x-axis on a logarithmic scale (10–10,000 ppm) and the y-axis representing enzyme inhibition. The fitted curve suggests dose-dependent inhibition, where higher concentrations lead to greater AChE inhibition. At lower concentrations, around 100 ppm, inhibition is minimal, suggesting that VGE has weak effect on AChE at these levels. As the concentration increases, between 100 and 1000 ppm, the percentage of inhibition gradually rises, indicating a stronger interaction between VGE and the enzyme. Moreover, the non-sigmoidal shape may indicate that the inhibition is still increasing at high concentrations, suggesting that the maximum effect has not been observed within the tested range.

7. Comparative Analyses of V. globosa Inhibitory Activities

The independent t-test revealed a significant difference ($p < 0.05$) between the DPPH inhibition activities of ethyl acetate fraction of *V. globosa* and Ascorbic acid, with ascorbic acid demonstrating significantly higher inhibition compared to the ethyl acetate flavonoid fraction.

Similarly, the independent t-test revealed a statistically significant difference ($p < 0.05$) in the cupric reducing antioxidant capacity between the ethyl acetate fraction of *V. globosa* and Ascorbic acid. Ascorbic acid demonstrates significantly higher antioxidant activity than the Ethyl acetate Flavonoid fraction. This highlights the stronger ability of ascorbic acid to reduce cupric ions, making it a more potent antioxidant in this assay.

For acetylcholinesterase (AChE) inhibition, analysis of variance (ANOVA) revealed that there is a significant difference ($p < 0.05$) between the plant sample and the standard. Galantamine demonstrates substantially higher inhibition activity compared to the Ethyl acetate fraction. Given the established role of Galantamine as a potent AChE inhibitor used in Alzheimer's treatment, these results are consistent with its expected bioactivity. In contrast, while the ethyl acetate fraction exhibits some inhibitory effect, it is significantly weaker in activity in comparison to Galantamine. Consequently, in the Post Hoc Analysis using Tukey's HSD test, multiple pairwise comparisons showed significant differences particularly between higher concentrations of Voacanga globosa (450ppm, 500 ppm, and 1000ppm) and the lower concentrations. Overall, this indicates a dose-dependent effect, with higher concentrations significantly differing from the lower concentrations, supporting the presence of a meaningful treatment effect.

CHAPTER 5

Summary, Conclusion, and Recommendations

This section summarizes the key findings, highlights the conclusions drawn from the study, and provides practical recommendations from researchers for future research and applications.

Summary of Findings

Based on the findings of the study, the following key points are enumerated:

1. The *Voacanga globosa* crude methanolic extract, besides its hexane, ethyl acetate, and aqueous fractions, resulted in brownish-green sticky masses soluble generally in alcohol and their respective solvents. At 8.22%, the crude methanolic extract (VGM) had its highest extraction yield. For the hexane fraction (VGH), yields were at 0.07%; for the ethyl acetate fraction (VGE), yields were at 0.73%. Meanwhile, for the aqueous fraction (VGA), yields were at 1.44%.
2. Confirmatory Test for secondary metabolites indicated the presence of alkaloids, flavonoids, tannins, cardiac glycosides, and saponins in the Crude methanolic extract as well as in fractions of *Voacanga globosa*.

3. FTIR analysis, which was used to analyze the crude extract and fractions, revealed that the ethyl acetate fraction displayed the most defined peaks in relation to flavonoids as well as phenolic compounds.
4. The Total Flavonoid and Total Phenolic Content showed the highest amount within the ethyl acetate fraction of *Voacanga globosa* among the tested fractions, suggesting that it is the optimal solvent for the extracting of flavonoid-rich bioactive compounds from *Voacanga globosa* leaves. These results support the potential of Voacanga globosa leaves to be used in antioxidant as well as acetylcholinesterase applications.
5. The results show that the DPPH radical scavenging and the cupric reducing antioxidant activities of the plant sample have a significant difference from those of the positive control. The low inhibition of the plant sample, relative to the positive control, is indicative of its weak potential antioxidant capacity. This may be accounted for by the diversity in the bioactive compounds' nature and composition of flavonoids and phenolics in all of which, on behalf of the plant sample, could have impacted its capacity to donate the hydrogen, neutralize the DPPH radicals, and its electron-donating capacity.
6. *Voacanga globosa*, tested at ten different concentrations, demonstrated a significant difference in acetylcholinesterase (AChE) inhibition compared to the positive control. The inhibition followed a dose-dependent pattern, with higher concentrations leading to greater inhibition. However, no concentration of the plant sample achieved inhibition levels comparable to Galantamine, indicating that while the extract exhibits potential AChE inhibitory activity, it is less potent than the pharmaceutical standard.
7. Comparative analyses revealed significant differences ($p < 0.05$) in the inhibitory activities of *Voacanga globosa* and positive controls, with ascorbic acid showing superior DPPH scavenging and cupric reducing antioxidant capacity, while galantamine exhibited significantly higher acetylcholinesterase (AChE) inhibition. Post hoc analysis confirmed a dose-dependent effect in *Voacanga globosa* at higher concentrations.

CONCLUSION

The *Voacanga globosa* ethyl acetate fraction exhibited antioxidant and acetylcholinesterase inhibitory activities, although with lower potency than the positive controls. The DPPH radical scavenging activity and Cupric reducing antioxidant capacity suggest the presence of bioactive compounds, albeit with mild antioxidant properties. Additionally, its dose-dependent acetylcholinesterase inhibition indicates potential neuroprotective effects, though it remains less active than Galantamine. This resulted in the rejection of the null hypotheses (Ho1 and Ho2), indicating a significant difference ($p<0.05$) between Voacanga globosa and the positive controls. Further studies on compound isolation and characterization are needed to better understand its mechanisms and therapeutic potential.

Recommendations

1. Investigate other parts of *Voacanga globosa*, such as its fruits and roots, for their potential flavonoid content and evaluate their antioxidant and acetylcholinesterase inhibitory activities.
2. Optimize and explore other extraction methods, such as microwave-assisted extraction, to extract flavonoids and enhance yield.
3. Expand the range of concentration in assays to assess whether they exhibit higher activity that is comparable with the positive controls.
4. Characterize flavonoids from *Voacanga globosa* using LC-MS, and NMR spectroscopy.
5. Explore the potential synergistic effects of flavonoids and other constituents such as alkaloids present in Voacanga globosa on its antioxidant and acetylcholinesterase inhibitory activities.

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APPENDIX A

Authentication of Bayag-Usa (*Voacanga globosa)*

Department of Agriculture
BUREAU OF PLANT INDUSTRY
Crop Research and Production Support Division
692 San Andres Street, Malate, Manila, Philippines
Email Address: crpsd@buplant.da.gov.ph
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ISO 9001:2015 Certified
Quality Management System

PLT-ID-CRPSD-557-2024
August 20, 2024

PLANT VERIFICATION/CERTIFICATION

1. Local/Common Name:	Bayag-Usa (Tag.), Testicle Tree (Engl.)
2. Family / Scientific Name:	APOCYNACEAE / *Voacanga globosa (Blanco) Merr.*
3. Collector's Name & Address:	Xeminesse Krixette C. Magbanua *et al.* Centro Escolar University – Manila 9 Mendiola St., San Miguel, Manila City.
4. Collection Site/Source:	Laguna
5. Date of Collection:	August 10, 2024
6. Types of Sample:	( ) Whole plant ( ) Leaves ( ) Stem ( ) Roots ( ) Flowers (X) Fruits ( ) Seeds ( ) Others:
7. Status of Sample:	Semi-Dried
8. Description/Remarks:	See attached sheet
9. References:	See attached sheet
10.Note:	For research purposes only

Verified and Certified by:

ALEX S. PEDROSO
Project Assistant II

Noted by:

MARK CHRISTOPHER E. VALDEZ
OIC, Crop Improvement and Plant Genetic Resources Section

Approved by:

CHRISTOPHER S. CRUZ
OIC, CRPSD

EFFECTIVITY DATE: AUGUST 15, 2023
FORM NO: BPI-QMS-KMT-F1
REVISION NO.: 5

www.buplant.da.gov.ph /BureauOfPlantIndustry

Family Name : APOCYNACEAE

Common Name : Bayag-Usa (Tag.), Testicle Tree (Engl.)

Scientific Name : *Voacanga globosa (Blanco) Merr.*

DESCRIPTION/REMARKS

Bayag-usa is a tree reaching a height of up to 3 meters. Leaves are simple, opposite, oblong-elliptic; dark green in the upper surface and pale beneath. Flowers are creamy white and showy. Fruit comes in pairs, brownish, 3 to 5 centimeters in diameter. Seeds are embedded in the pulp.

Endemic to the Philippines. In secondary forests and thickets at lower elevations. Sometimes ornamentally cultivated for its flowers. Study identified a bisindole alkaloid from V. grandifolia, Voacinol, assigned a 14,14'-methylene-bis-18-hydroxytabersonine structure on the basis of spectral data. Preliminary phytochemical screening of leaves yielded alkaloids, saponins, 2-deoxysugars, and hydrolyzable tannins. A bioassay-guided purification yielded Globospiramine, a new spirobisindole alkaloid possessing an Aspidosperma-Aspidosperma skeleton, together with deoxyvobtusine, deoxyvobtusine lactone, vobtusine lactone and lupeol.

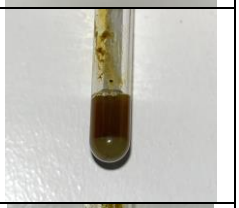
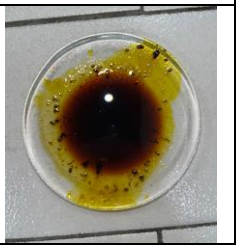
Reference:

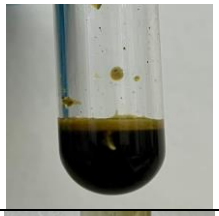
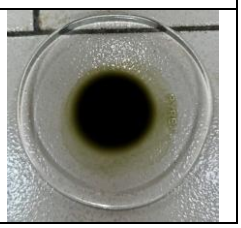
Stuart G.U. Jr., M.D. Updated January 2024. Bayag-usa (*Voacanga globosa (Blanco) Merr.*) Philippine Medicinal Plant. Stuart XChange.http://www.stuartxchange.org

APPENDIX B

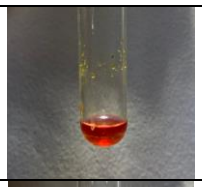
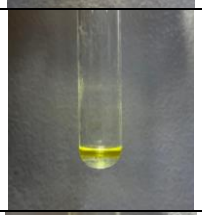
Phytochemical Tests

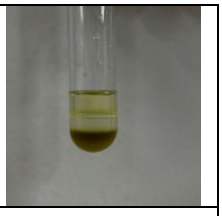
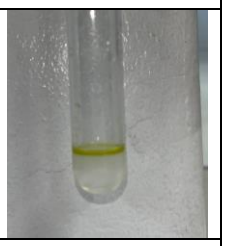
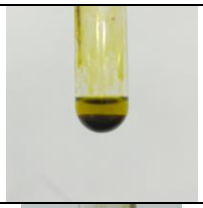
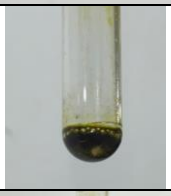
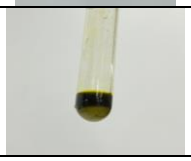
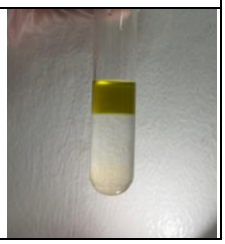
Phytochemical Test on Crude Methanolic Extract

TEST	EXPECTED RESULT	ACTUAL RESULT	PICTURES
ALKALOIDS			
Wagner's Test	Yellow-brown ppt	Yellow-brown ppt (Positive)	
Mayer's Test	Creamy/ white colored ppt	Creamy/ white colored ppt (Positive)	
Dragendroff's Test	Orange- Red ppt	Orange- Red ppt (Positive)	
GLYCOSIDES			
Cardiac glycoside Keller Killiani	Reddish brown ring	Greenish black (Negative)	
Anthraquinone Borntragers	Pink to red color	Dark brown solution (Negative)	
SAPONINS			
Froth Test	1cm layer of foam	1cm layer of foam (positive)	
FLAVONOIDS			
Alkaline Reagent Test	Intense yellow color	Intense yellow color (positive)	

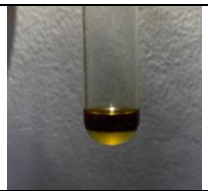
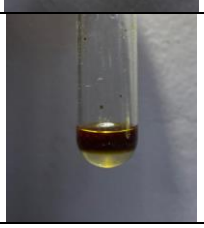
Shinoda	Pink reddish brown color	Greenish black solution (negative)	
Lead Acetate	Yellow ppt	Yellow ppt (Positive)	
TANNINS			
FeCl Test	Nonhydrolyzable: brownish-green Hydrolyzable: bluish black	Brownish-green (Positive - Nonhydrolyzable)	

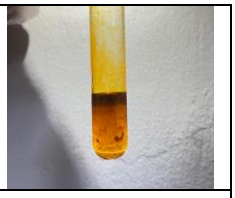
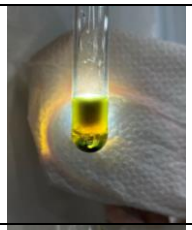
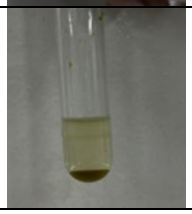
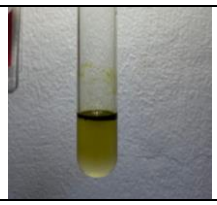
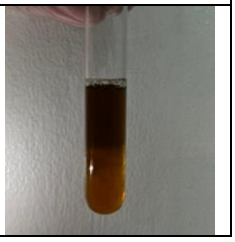
Phytochemical Test on n-hexane Fraction

TEST	EXPECTED RESULT	ACTUAL RESULT	PICTURES
ALKALOIDS			
Wagner's Test	Yellow-brown ppt	Red orange solution (Negative)	
Mayer's Test	Creamy/ white colored ppt	Yellow upper layer Colorless lower layer (Negative)	
Dragendroff's Test	Orange- Red ppt	Orange Solution (Negative)	
GLYCOSIDES			
Cardiac glycoside Keller Killiani	Reddish brown ring	Reddish brown ring (Positive)	

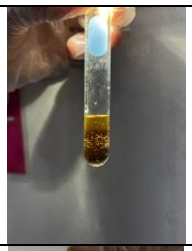
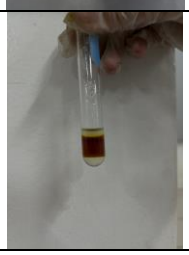
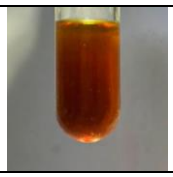
Anthraquinone Borntragers	Pink to red color	Colorless upper layer Green lower layer (Negative)	
SAPONINS			
Froth Test	1cm layer of foam	Colorless Solution (Negative)	
FLAVONOIDS			
Alkaline Reagent Test	Intense yellow color	Dark green ppt (Negative)	
Shinoda	Pink reddish brown color	Dark green solution (Negative)	
Lead Acetate	Yellow ppt	Yellow ppt (positive)	
TANNINS			
FeCl Test	Nonhydrolyzable: brownish-green Hydrolyzable: bluish black	Light green upper layer Colorless lower layer (Negative)	

Phytochemical Test on Ethyl Acetate Fraction

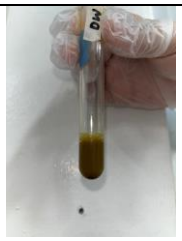
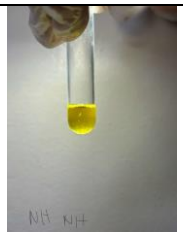
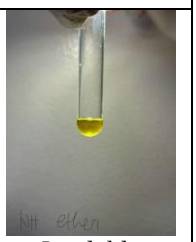
TEST	EXPECTED RESULT	ACTUAL RESULT	PICTURES
ALKALOIDS			
Wagner's Test	Yellow-brown ppt	Dark green upper layer Colorless lower layer (Negative)	
Mayer's Test	Creamy/ white colored ppt	Dark green upper layer Colorless lower layer (Negative)	

Dragendroff's Test	Orange- Red ppt	Orange Solution (Negative)	
GLYCOSIDES			
Cardiac glycoside Keller Killiani	Reddish brown ring	Reddish brown ring (positive)	
Anthraquinone Borntragers	Pink to red color	Green ppt (Negative)	
SAPONINS			
Froth Test	1cm layer of foam	Slightly turbid solution (Negative)	
FLAVONOIDS			
Alkaline Reagent Test	Intense yellow color	Intense yellow color (positive)	
Shinoda	Pink reddish brown color	Pink reddish brown color (Positive)	
Lead Acetate	Yellow ppt	Yellow ppt (positive)	
TANNINS			
FeCl Test	Nonhydrolyzable: brownish-green Hydrolyzable: bluish black	Dark brown solution (Negative)	

Phytochemical Test on Aqueous Fraction

TEST	EXPECTED RESULT	ACTUAL RESULT	PICTURES
ALKALOIDS			
Wagner's Test	Yellow-brown ppt	Orange solution (Negative)	
Mayer's Test	Creamy/ white colored ppt	Light orange solution (Negative)	
Dragendroff's Test	Orange- Red ppt	Orange solution (Negative)	
GLYCOSIDES			
Cardiac glycoside Keller Killiani	Reddish brown ring	Reddish brown ring (Positive)	
Anthraquinone Borntragers	Pink to red color	Brown upper layer Colorless lower layer (Negative)	
SAPONINS			
Froth Test	1 cm layer of foam	1 cm layer of foam (positive)	
FLAVONOIDS			
Alkaline Reagent Test	Intense yellow color	Orange brown solution (Negative)	

Shinoda	Pink reddish brown color	Brown solution with 1 cm layer of foam (Negative)	
Lead Acetate	Yellow ppt	Yellow ppt (Positive)	
TANNINS			
FeCl Test	Nonhydrolyzable: brownish-green Hydrolyzable: bluish black	Non Hydrolyzable: brownish -green	

	SOLUBILITY TEST				
	DISTILLED WATER	**N-HEXANE**	**CHLOROFORM**	**ALCOHOL**	**ETHER**
CRUDE METHANOLIC EXTRACT	 Soluble	 Insoluble	 Insoluble	 Soluble	 Insoluble
ETHYL ACETATE	 Insoluble	 Insoluble	 Insoluble	 Soluble	 Insoluble
N-HEXANE	 Insoluble	 Soluble	 Soluble	 Insoluble	 Insoluble
AQUEOUS	 Soluble	 Insoluble	 Insoluble	 Soluble	 Insoluble

APPENDIX C

Statistical Analysis and Computations

Percentage Yield	$Percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ plant\ sample}\ x\ 100$
Total Flavonoid Content	$TFC\ (QE\ mg/g) = C * DF * V/m$
Total Phenolic Content	$TPC\ (GAE\ mg/g) = C * DF * V/m$
CUPRAC %Inhibition	$\%\ Inhibition = \left[\frac{Absorbance_{(-)\ control} - Absorbance_{sample}}{Absorbance_{(-)\ control}}\right] \times 100$
AChE %Inhibition	$\%\ Inhibition = \left[\frac{Absorbance_{(-)\ control} - Absorbance_{sample}}{Absorbance_{(-)\ control}}\right] \times 100$

Percentage Yield of Samples

$Percentage\ Yield\ (\%) = \frac{weight\ of\ dried\ extract}{weight\ of\ dried\ plant\ sample} \times 100$	
VGM	$\frac{74.4g}{905.4g} \times 100 = 8.22\%$
VGH	$\frac{0.70g}{905.4g} \times 100 = 0.07\%$
VGE	$\frac{6.6g}{905.4g} \times 100 = 0.73\%$
VGA	$\frac{13.0g}{905.4g} \times 100 = 1.44\%$

Raw Data of Assays

Raw Data for the *Total Phenolic and Flavonoid Content of Crude Methanolic Extract, Hexane Fraction, Ethyl Acetate Fraction, and Aqueous Fraction of Voacanga globosa.*

Table. Total Phenolic Content

Sample	TPC (mg GAE/mL)
VG MetOH Extract	13.92 ± 0.97
VG Hexane Fraction	1.79 ± 0.04
VG EtAc Fraction	24.78 ± 1.39
VG Aqueous Fraction	12.40 ± 0.80

Table. Total Flavonoid Content

Sample	TFC (ug GAE/mL)
VG MetOH Extract	2.20 ± 0.04
VG Hexane Fraction	0.59 ± 0.03
VG EtAc Fraction	5.14 ± 0.39
VG Aqueous Fraction	0.68 ± 0.06

Raw Data for DPPH radical scavenging activity of ethyl acetate fraction of Voacanga globosa and ascorbic acid

DPPH Radical Scavenging Activity, % Inhibition as Absorbance (Mean ± SD)					
Sample ($\mu g/\mu L$)	10	15	20	25	30
VGE	2.61 ± 0.20*	4.64 ± 0.24*	5.31 ± 0.09*	7.09 ±0.32*	8.53 ±0.26*
Ascorbic acid	19.72 ± 0.60	31.12 ±0.20	41.37 ±0.08	48.83 ±0.12	63.07 ±0.72

Table. DPPH Radical Scavenging Activity (% Inhibition)

	At 10 ug/uL		At 15 ug/uL		At 20 ug/uL		At 25 ug/uL		At 30 ug/uL	
Trial	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid
1	2.4	19.12	4.4	31.32	5.2	41.29	6.8	48.71	8.24	63.79
2	2.64	20.31	4.64	30.92	5.36	41.45	7.04	48.95	8.64	62.35
3	2.8	19.72	4.88	31.12	5.36	41.37	7.44	48.83	8.72	63.07
Mean	**2.61**	**19.72**	**4.64**	**31.12**	**5.31**	**41.37**	**7.09**	**48.83**	**8.53**	**63.07**
SD	0.20	0.60	0.24	0.20	0.09	0.08	0.32	0.12	0.26	0.72

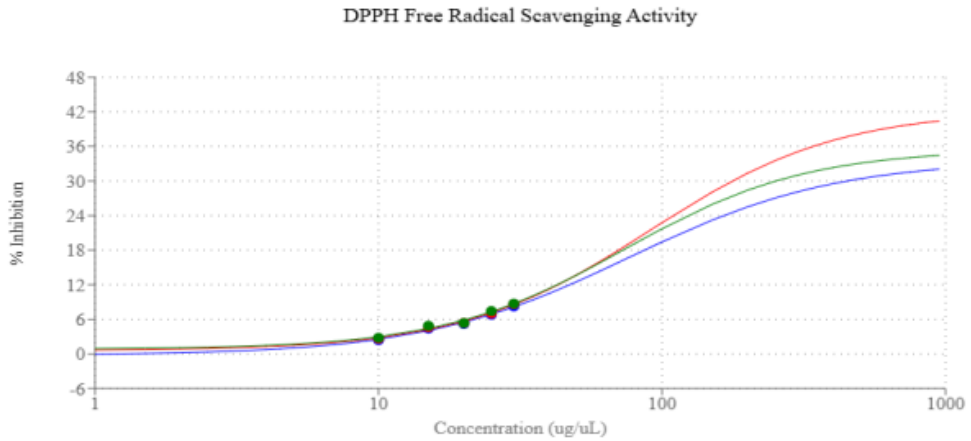
Table. DPPH Radical Scavenging Activity (IC50)

GROUP	IC50 (ug/uL)
VG EtAc Fraction	80.10 ± 9.14
Ascorbic acid	80.45 ± 5.85

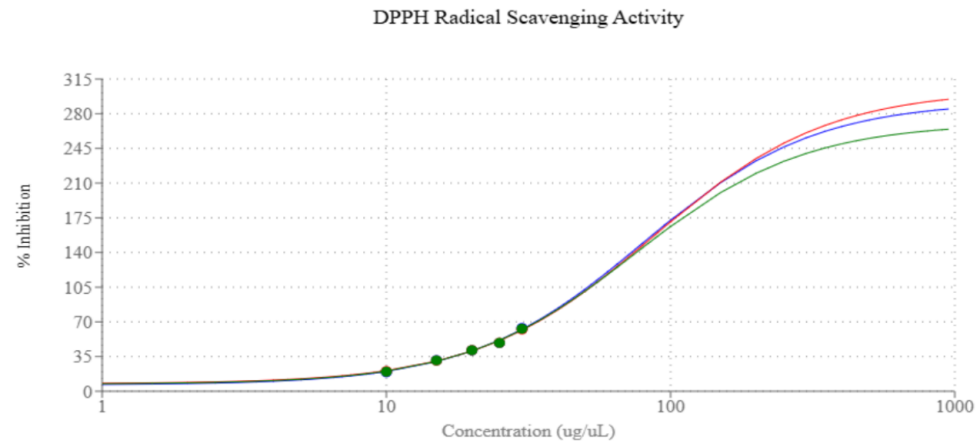
**vs ascorbic acid (positive control), p<0.05 is considered statistically significant (Independent t-test)*

Trial	VG EtAc Fraction	Ascorbic acid
1	76.16	79.78
2	90.55	86.60
3	73.59	74.96
Mean	**80.10**	**80.45**
SD	9.14	5.85

IC50 - VG EtAc Fraction



IC50 -Ascorbic Acid



IC50 - VG EtAc Fraction

IC_{50} Regression Results [t1]

Parameter	Value
IC_{50}	76.1587
Equations	(show alternative)
Equation	$Y = -0.2248 + \frac{33.6251 + 0.2248}{1 + (\frac{X}{76.1587})^{-1.194}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t2]

Parameter	Value
IC_{50}	90.547
Equations	(show alternative)
Equation	$Y = 0.5832 + \frac{42.2546 - 0.5832}{1 + (\frac{X}{90.547})^{-1.3075}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t3]

Parameter	Value
IC_{50}	73.5889
Equations	(show alternative)
Equation	$Y = 0.8236 + \frac{35.4577 - 0.8236}{1 + (\frac{X}{73.5889})^{-1.3618}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC50 -Ascorbic Acid

IC_{50} Regression Results [t1]

Parameter	Value
IC_{50}	79.7834
Equations	(show alternative)
Equation	$Y = 6.3023 + \frac{292.585 - 6.3023}{1 + (\frac{X}{79.7834})^{-1.443}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t2]

Parameter	Value
IC_{50}	86.5953
Equations	(show alternative)
Equation	$Y = 7.4984 + \frac{304.2148 - 7.4984}{1 + (\frac{X}{86.5953})^{-1.4184}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t3]

Parameter	Value
IC_{50}	74.9574
Equations	(show alte
Equation	$Y = 7.1137 + \frac{270.6575 - 7.1137}{1 + (\frac{X}{74.9574})^{-1.4603}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

At 10 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	2.4	19.12
Max	2.8	20.31
Sum	7.84	59.15
Mean	2.613333	19.71667
Std. error	0.1162373	0.3435275
Variance	0.04053333	0.3540333
Stand. dev	0.2013289	0.595007
Median	2.64	19.72
25 prcntil	2.4	19.12
75 prcntil	2.8	20.31
Mode	NA	NA
Skewness	-0.5855827	-0.025209
Kurtosis	-2.333333	-2.333333
Geom. mean	2.608101	19.71068
Harm. mean	2.602817	19.70469
Coeff. var	7.703913	3.017787

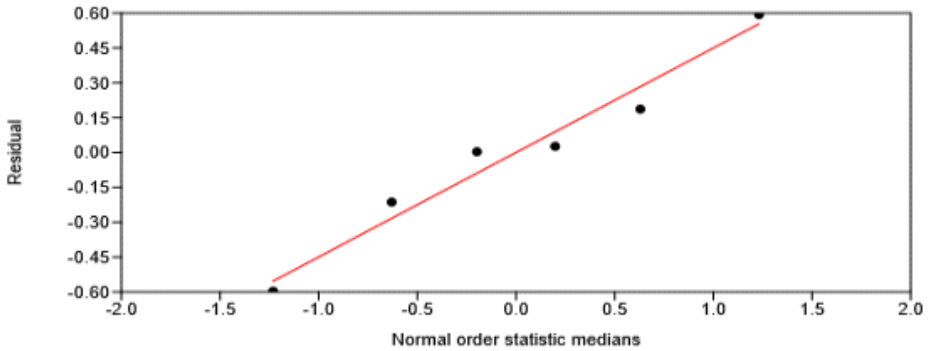
At 10 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.9868	1
p(normal)	0.7804	0.9907
Anderson-Darling A	0.2042	0.1895
p(normal)	0.5652	0.6306
p(Monte Carlo)	0.7826	0.9915
Lilliefors L	0.2194	0.1754
p(normal)	0.8367	1
p(Monte Carlo)	0.7752	0.9922
Jarque-Bera JB	0.3098	0.2813
p(normal)	0.8565	0.8688
p(Monte Carlo)	0.7788	0.9902

Residuals

Shapiro-Wilk W	0.9802
p(normal)	0.9524



At 10 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	2.6133	Mean:	19.717		
95% conf.:	(2.1132 3.1135)	95% conf.:	(18.239 21.195)		
Variance:	0.040533	Variance:	3.54E-01		
Difference between means:	17.103				
95% conf. interval (parametric):	(16.096 18.11)				
95% conf. interval (bootstrap):	(16.51 17.687)				
t :	47.161	p (same mean):	1.21E-06	Critical t value (p	2.7764
Uneq. var. t :	47.161	p (same mean):	0.00010899	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.106			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	2979	Cohen´s D:	38.51		
Decisive evidence for unequal means	Huge effect size				

At 15 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	4.4	30.92
Max	4.88	31.32
Sum	13.92	93.36
Mean	4.64	31.12
Std. error	0.1385641	0.1154701
Variance	0.0576	0.04
Stand. dev	0.24	0.2
Median	4.64	31.12
25 prcntil	4.4	30.92
75 prcntil	4.88	31.32
Mode	NA	NA
Skewness	1.65E-14	-1.60E-13
Kurtosis	-2.333333	-2.333333
Geom. mean	4.635858	31.11957
Harm. mean	4.631717	31.11914
Coeff. var	5.172414	0.6426735

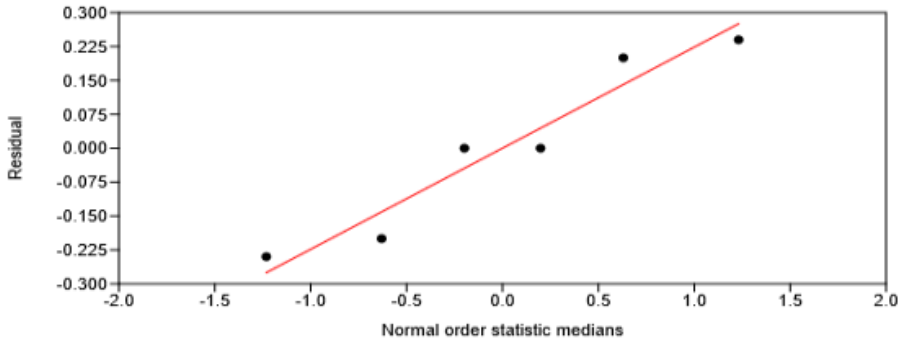
At 15 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	1	1
p(normal)	1	1
Anderson-Darling A	0.1895	0.1895
p(normal)	0.6307	0.6307
p(Monte Carlo)	0.9999	1
Lilliefors L	0.1747	0.1747
p(normal)	1	1
p(Monte Carlo)	1	1
Jarque-Bera JB	0.2813	0.2813
p(normal)	0.8688	0.8688
p(Monte Carlo)	1	1

Residuals

Shapiro-Wilk W	0.9076
p(normal)	0.4209



At 15 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	4.64	Mean:	31.12		
95% conf.:	(4.0438 5.2362)	95% conf.:	(30.623 31.617)		
Variance:	0.0576	Variance:	4.00E-02		
Difference between means:	26.48				
95% conf. interval (parametric):	(25.979 26.981)				
95% conf. interval (bootstrap):	(26.187 26.773)				
t :	146.81	p (same mean):	1.29E-08	Critical t value (p	2.7764
Uneq. var. t :	146.81	p (same mean):	2.11E-08	Critical t value (p	2.8114
Monte Carlo permutation:	p (same mean):	0.1048			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	7.46E+04	Cohen´s D:	119.9		
Decisive evidence for unequal means	Huge effect size				

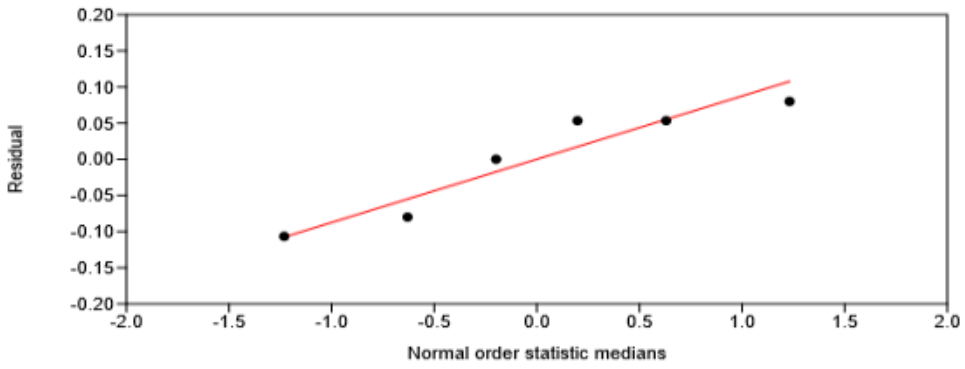
At 20 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.75	1
p(normal)	0	1
Anderson-Darling A	0.4878	0.1895
p(normal)	0.05651	0.6307
p(Monte Carlo)	0.0001	1
Lilliefors L	0.3848	0.1747
p(normal)	0.08879	1
p(Monte Carlo)	0.0001	1
Jarque-Bera JB	0.5313	0.2813
p(normal)	0.7667	0.8688
p(Monte Carlo)	0.0001	1

Residuals

Shapiro-Wilk W	0.8804
p(normal)	0.2709



At 20 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	5.2	41.29
Max	5.36	41.45
Sum	15.92	124.11
Mean	5.306667	41.37
Std. error	0.05333333	0.04618802
Variance	0.008533333	0.0064
Stand. dev	0.09237604	0.08
Median	5.36	41.37
25 prcntil	5.2	41.29
75 prcntil	5.36	41.45
Mode	5.36	NA
Skewness	-1.732051	4.00E-13
Kurtosis	-2.333333	-2.333333
Geom. mean	5.306127	41.36995
Harm. mean	5.305584	41.3699
Coeff. var	1.740755	0.1933768

At 20 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	5.306667	Mean:	41.37		
95% conf.:	(2.1132 3.1135)	95% conf.:	(18.239 21.195)		
Variance:	0.040533	Variance:	3.54E-01		
Difference between means:	36.063333				
95% conf. interval (parametric):	(16.096 18.11)				
95% conf. interval (bootstrap):	(16.51 17.687)				
t :	47.161	p (same mean):	1.21E-06	Critical t value (p	2.7764
Uneq. var. t :	47.161	p (same mean):	0.00010899	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.106			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	2979	Cohen´s D:	38.51		
Decisive evidence for unequal means	Huge effect size				

At 25 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	6.8	48.71
Max	7.44	48.95
Sum	21.28	146.49
Mean	7.093333	48.83
Std. error	0.1866667	0.06928203
Variance	0.1045333	0.0144
Stand. dev	0.3233162	0.12
Median	7.04	48.83
25 prcntil	6.8	48.71
75 prcntil	7.44	48.95
Mode	NA	NA
Skewness	0.7221086	-2.66E-13
Kurtosis	-2.333333	-2.333333
Geom. mean	7.088454	48.8299
Harm. mean	7.08361	48.8298
Coeff. var	4.558028	0.2457506

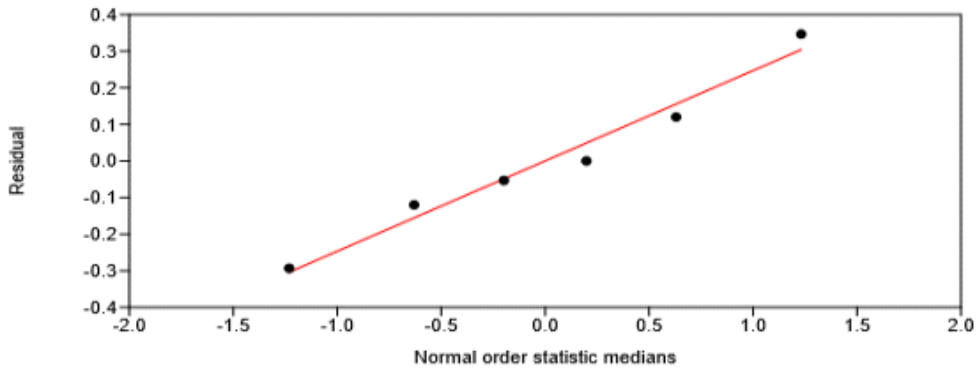
At 25 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.9796	1
p(normal)	0.7262	1
Anderson-Darling A	0.2123	0.1895
p(normal)	0.5359	0.6307
p(Monte Carlo)	0.7307	1
Lilliefors L	0.2322	0.1747
p(normal)	0.7715	1
p(Monte Carlo)	0.7236	1
Jarque-Bera JB	0.3247	0.2813
p(normal)	0.8501	0.8688
p(Monte Carlo)	0.7301	1

Residuals

Shapiro-Wilk W	0.9831
p(normal)	0.9659



At 25 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	7.0933	Mean:	48.83		
95% conf.:	(6.2902 7.8965)	95% conf.:	(48.532 49.128)		
Variance:	0.10453	Variance:	1.44E-02		
Difference between means:	41.737				
95% conf. interval (parametric):	(41.184 42.289)				
95% conf. interval (bootstrap):	(41.443 42.07)				
t :	209.62	p (same mean):	3.11E-09	Critical t value (p	2.7764
Uneq. var. t :	209.62	p (same mean):	1.88E-06	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.098			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	1.81E+05	Cohen´s D:	171.2		
Decisive evidence for unequal means	Huge effect size				

At 30 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	8.24	62.35
Max	8.72	63.79
Sum	25.6	189.21
Mean	8.533333	63.07
Std. error	0.1484737	0.4156922
Variance	0.06613333	0.5184
Stand. dev	0.257164	0.72
Median	8.64	63.07
25 prcntil	8.24	62.35
75 prcntil	8.72	63.79
Mode	NA	NA
Skewness	-1.545393	0.00E+00
Kurtosis	-2.333333	-2.333333
Geom. mean	8.530722	63.06726
Harm. mean	8.528085	63.06452
Coeff. var	3.013641	1.141589

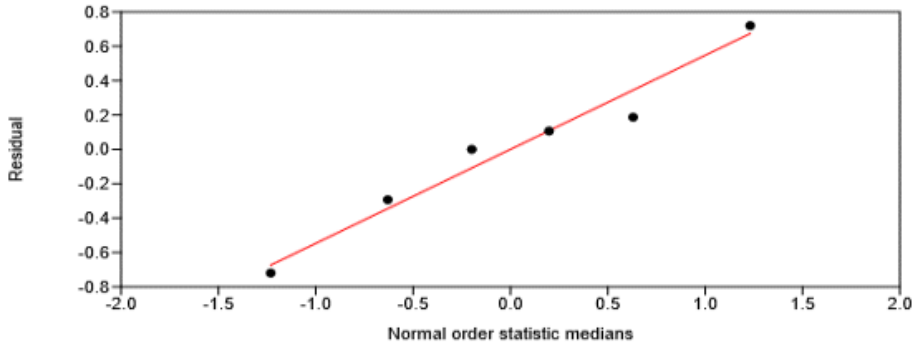
At 30 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.871	1
p(normal)	0.2983	1
Anderson-Darling A	0.3382	0.1895
p(normal)	0.2003	0.6307
p(Monte Carlo)	0.2959	1
Lilliefors L	0.3275	0.1747
p(normal)	0.2541	1
p(Monte Carlo)	0.2978	1
Jarque-Bera JB	0.4803	0.2813
p(normal)	0.7865	0.8688
p(Monte Carlo)	0.2956	1

Residuals

Shapiro-Wilk W	0.9794
p(normal)	0.9485



At 30 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	8.5333	Mean:	63.07		
95% conf.:	(7.8945 9.1722)	95% conf.:	(61.281 64.859)		
Variance:	0.066133	Variance:	5.18E-01		
Difference between means:	54.537				
95% conf. interval (parametric):	(53.311 55.762)				
95% conf. interval (bootstrap):	(53.817 55.23)				
t :	123.55	p (same mean):	2.57E-08	Critical t value (p	2.7764
Uneq. var. t :	123.55	p (same mean):	8.41E-06	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.1019			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	4.70E+04	Cohen´s D:	100.9		
Decisive evidence for unequal means	Huge effect size				

IC50

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	73.59	74.96
Max	90.55	86.6
Sum	240.3	241.34
Mean	80.1	80.44667
Std. error	5.277408	3.376672
Variance	83.5531	34.20573
Stand. dev	9.140738	5.848567
Median	76.16	79.78
25 prcntil	73.59	74.96
75 prcntil	90.55	86.6
Mode	NA	NA
Skewness	1.579291	5.06E-01
Kurtosis	-2.333333	-2.333333
Geom. mean	79.76482	80.30585
Harm. mean	79.44352	80.16619
Coeff. var	11.41166	7.270117

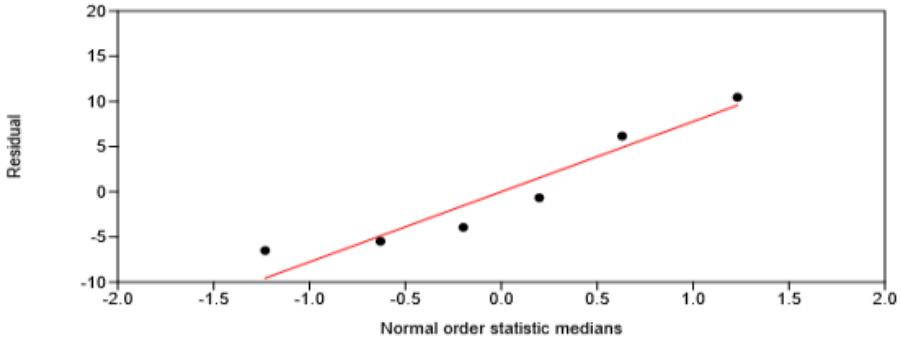
IC50

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.8607	0.9903
p(normal)	0.2694	0.8112
Anderson-Darling A	0.3505	0.2004
p(normal)	0.1803	0.581
p(Monte Carlo)	0.2711	0.812
Lilliefors L	0.3334	0.212
p(normal)	0.2307	0.8695
p(Monte Carlo)	0.2652	0.8099
Jarque-Bera JB	0.4891	0.3026
p(normal)	0.7831	0.8596
p(Monte Carlo)	0.2683	0.8147

Residuals

Shapiro-Wilk W	0.8879
p(normal)	0.3075



IC50

Group Comparison Test

t tests for equal means

VG EtAc Fraction	Ascorbic acid		
N:	3	N:	3
Mean:	80.1	Mean:	80.447
95% conf.:	(57.393 102.81)	95% conf.:	(65.918 94.975)
Variance:	83.553	Variance:	3.42E+01

Difference between means:	0.34667				
95% conf. interval (parametric):	(-17.048 17.742)				
95% conf. interval (bootstrap):	(-9.1867 10.63)				
t :	0.055332	p (same mean):	9.59E-01	Critical t value (p	2.7764
Uneq. var. t :	0.055332	p (same mean):	9.59E-01	Critical t value (p	2.9777
Monte Carlo permutation:	p (same mean):	0.9897			
Exact permutation:	p (same mean):	0.95238			
Bayes factor:	5.63E-01	Cohen´s D:	0.04518		
No evidence for either equal or unequal r		Very small effect size			

Raw Data for Cupric reducing antioxidant capacity of ethyl acetate fraction of Voacanga globosa

Cupric reducing antioxidant capacity, CUPRAC (% Inhibition)

Cupric reducing antioxidant capacity, % Inhibition as Absorbance (Mean ± SD)					
Sample ($\mu g/\mu L$)	10	15	20	25	30
VG EtAc Fraction	0.07 ±0.00 *	0.12 ± 0.01 *	0.14 ± 0.00 *	0.20 ±0.00 *	0.25 ±0.00 *
Ascorbic acid	0.25 ± 0.00	0.37 ± 0.00	0.49 ±0.00	0.61 ±0.00	0.67 ±0.00

**vs ascorbic acid (positive control), p <0.05 is considered statistically significant (independent t-test)*

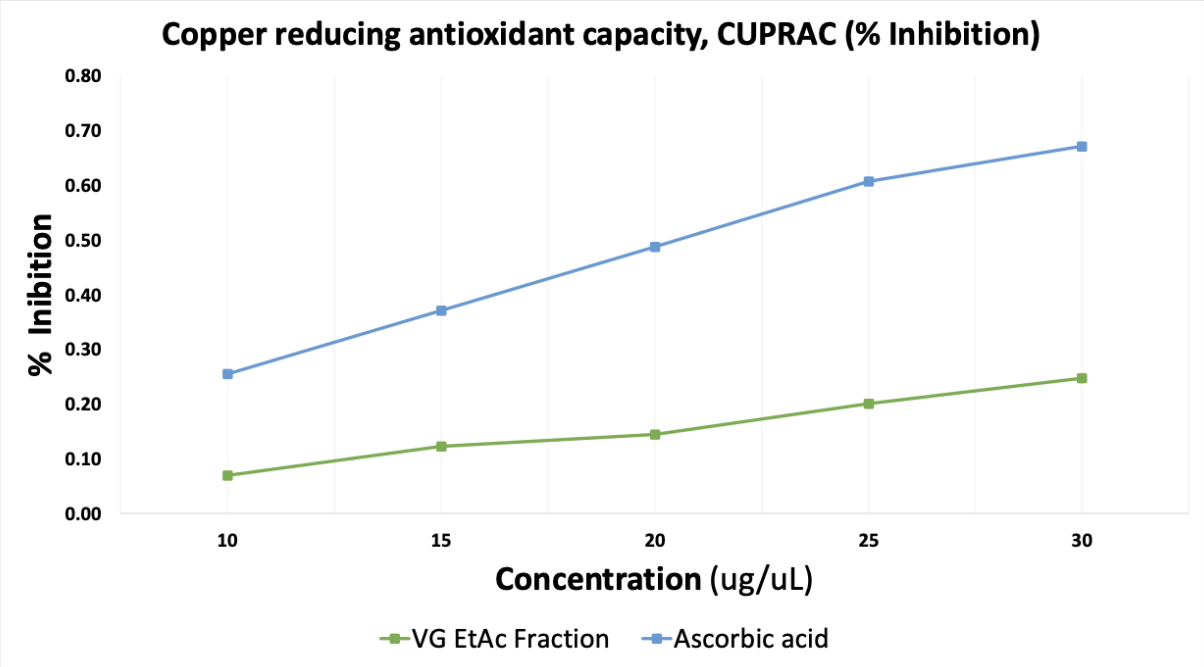
Table. Copper reducing antioxidant capacity, CUPRAC (% Inhibition)

GROUP	Copper reducing antioxidant capacity, % Inhibition as Absorbance (Mean ± SD)				
	At 10 ug/uL	**At 15 ug/uL**	**At 20 ug/uL**	**At 25 ug/uL**	**At 30 ug/uL**
VG EtAc Fraction	0.07 ± 0.00 *	0.12 ± 0.01 *	0.14 ± 0.00 *	0.20 ± 0.00 *	0.25 ± 0.00 *
Ascorbic acid	0.25 ± 0.00	0.37 ± 0.00	0.49 ± 0.00	0.61 ± 0.00	0.67 ± 0.00

**vs ascorbic acid (positive control), p<0.05 is considered statistically significant (Independent t-test)*

Table. Copper reducing antioxidant capacity, CUPRAC (% Inhibition)

	At 10 ug/uL		At 15 ug/uL		At 20 ug/uL		At 25 ug/uL		At 30 ug/uL	
Trial	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid	VG EtAc Fraction	Ascorbic acid
1	0.067	0.253	0.122	0.369	0.144	0.486	0.2	0.608	0.248	0.67
2	0.07	0.257	0.128	0.374	0.145	0.49	0.201	0.61	0.246	0.671
3	0.071	0.254	0.118	0.369	0.143	0.486	0.201	0.603	0.247	0.672
Mean	**0.07**	**0.25**	**0.12**	**0.37**	**0.14**	**0.49**	**0.20**	**0.61**	**0.25**	**0.67**
SD	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00



At 20 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	5.2	41.29
Max	5.36	41.45
Sum	15.92	124.11
Mean	5.306667	41.37
Std. error	0.05333333	0.04618802
Variance	0.008533333	0.0064
Stand. dev	0.09237604	0.08
Median	5.36	41.37
25 prcntil	5.2	41.29
75 prcntil	5.36	41.45
Mode	5.36	NA
Skewness	-1.732051	4.00E-13
Kurtosis	-2.333333	-2.333333
Geom. mean	5.306127	41.36995
Harm. mean	5.305584	41.3699
Coeff. var	1.740755	0.1933768

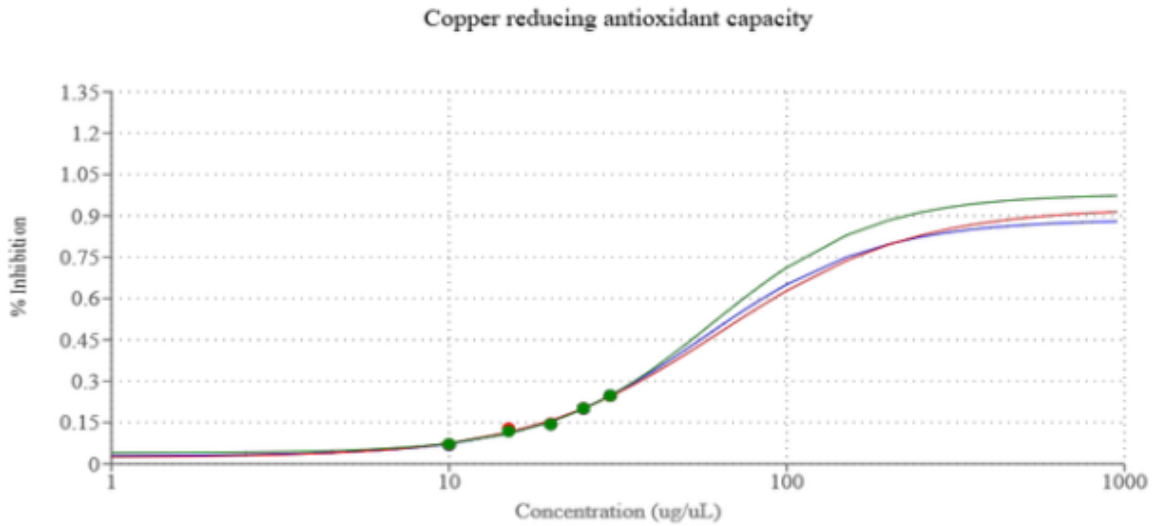
Table. Copper reducing antioxidant capacity, CUPRAC (IC50)

GROUP	IC50 (ug/uL)
VG EtAc Fraction	59.91 ± 3.30 *
Ascorbic acid	20.72 ± 0.42

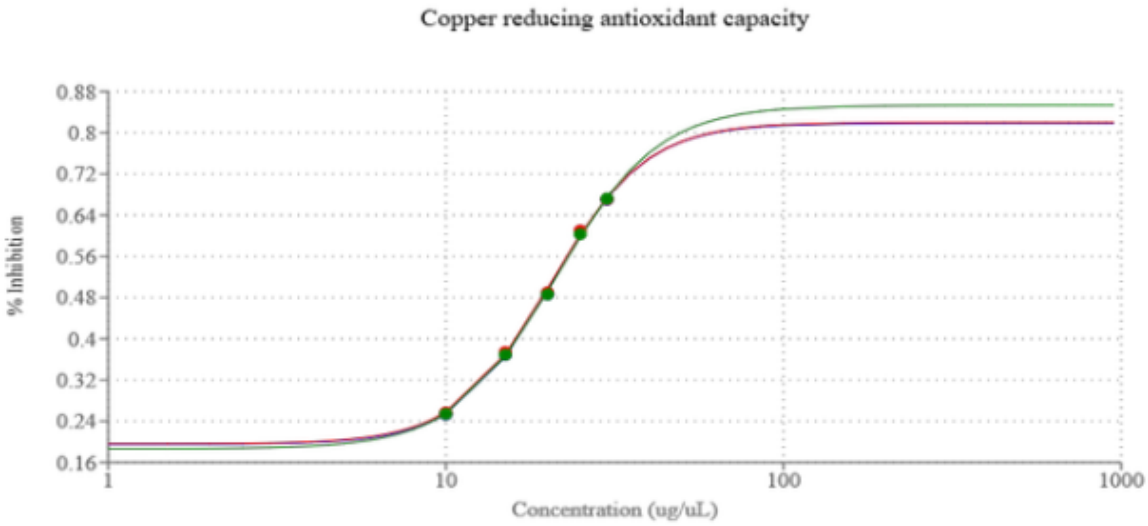
**vs ascorbic acid (positive control), p<0.05 is considered statistically significant (Independent t-test)*

Trial	VG EtAc Fraction	Ascorbic acid
1	56.57	20.52
2	63.16	20.43
3	60.01	21.20
Mean	**59.91**	**20.72**
SD	3.30	0.42

IC50 - VG EtAc Fraction



IC50 -Ascorbic Acid



IC50 - VG EtAc Fraction

IC_{50} Regression Results [t1]

Parameter	Value
IC_{50}	56.5731
Equations	(show alternative)
Equation	$Y = 0.0285 + \frac{0.8866 - 0.0285}{1 + (\frac{X}{56.5731})^{-1.7003}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t2]

Parameter	Value
IC_{50}	63.1563
Equations	(show alternative)
Equation	$Y = 0.0228 + \frac{0.9287 - 0.0228}{1 + (\frac{X}{63.1563})^{-1.5215}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t3]

Parameter	Value
IC_{50}	60.0058
Equations	(show alternative)
Equation	$Y = 0.039 + \frac{0.9785 - 0.039}{1 + (\frac{X}{60.0058})^{-1.8148}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC50 -Ascorbic Acid

IC_{50} Regression Results [t1]

Parameter	Value
IC_{50}	20.522
Equations	(show alternative)
Equation	$Y = 0.1949 + \frac{0.8184 - 0.1949}{1 + (\frac{X}{20.522})^{-3.1355}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t2]

Parameter	Value
IC_{50}	20.4259
Equations	(show alternative)
Equation	$Y = 0.1958 + \frac{0.8204 - 0.1958}{1 + (\frac{X}{20.4259})^{-3.0803}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

IC_{50} Regression Results [t3]

Parameter	Value
IC_{50}	21.1985
Equations	(show alternative)
Equation	$Y = 0.1863 + \frac{0.8531 - 0.1863}{1 + (\frac{X}{21.1985})^{-2.8855}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

At 10 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	0.067	0.253
Max	0.071	0.257
Sum	0.208	0.764
Mean	0.06933333	0.2546667
Std. error	0.00120185	0.00120185
Variance	4.33E-06	4.33E-06
Stand. dev	0.002081666	0.002081666
Median	0.07	0.254
25 prcntil	0.067	0.253
75 prcntil	0.071	0.257
Mode	NA	NA
Skewness	-1.293343	1.293343
Kurtosis	-2.333333	-2.333333
Geom. mean	0.06931231	0.254661
Harm. mean	0.06929111	0.2546554
Coeff. var	3.002403	0.8174081

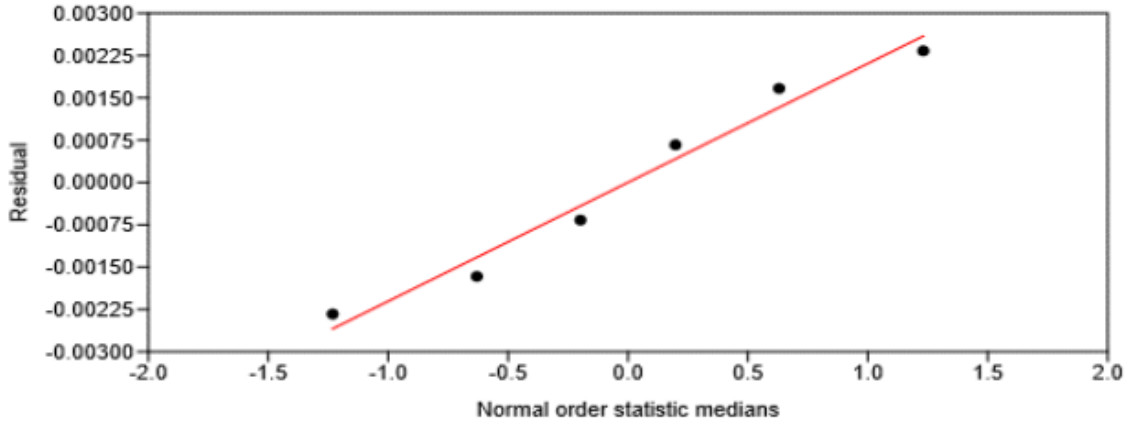
At 10 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.9231	0.9231
p(normal)	0.4633	0.4633
Anderson-Darling A	0.2769	0.2769
p(normal)	0.3337	0.3337
p(Monte Carlo)	0.463	0.4695
Lilliefors L	0.2923	0.2923
p(normal)	0.4228	0.4228
p(Monte Carlo)	0.4573	0.4627
Jarque-Bera JB	0.4206	0.4206
p(normal)	0.8103	0.8103
p(Monte Carlo)	0.4531	0.4555

Residuals

Shapiro-Wilk W	0.9481
p(normal)	0.7251



At 10 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	0.06933333	Mean:	0.2546667		
95% conf.:	(0.064162 0.0745(	95% conf.:	(0.2495 0.25984)		
Variance:	4.33E-06	Variance:	4.33E-06		
Difference between means:	0.18533337				
95% conf. interval (parametric):	(0.18061 0.19005)				
95% conf. interval (bootstrap):	(0.18267 0.188)				
t :	109.04	p (same mean):	4.24E-08	Critical t value (ɾ	2.7764
Uneq. var. t :	109.04	p (same mean):	4.24E-08	Critical t value (ɾ	2.7764
Monte Carlo permutation:	p (same mean):	0.0996			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	3.34E+04	Cohen´s D:	89.03		
Decisive evidence for unequal means	Huge effect size				

At 15 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	0.118	0.369
Max	0.128	0.374
Sum	0.368	1.112
Mean	0.1226667	0.3706667
Std. error	0.002905933	0.001666667
Variance	2.53E-05	8.33E-06
Stand. dev	0.005033223	0.002886751
Median	0.122	0.369
25 prcntil	0.118	0.369
75 prcntil	0.128	0.374
Mode	NA	0.369
Skewness	5.86E-01	1.73E+00
Kurtosis	-2.333333	-2.333333
Geom. mean	0.1225982	0.3706592
Harm. mean	0.12253	0.3706517
Coeff. var	4.103171	0.7787998

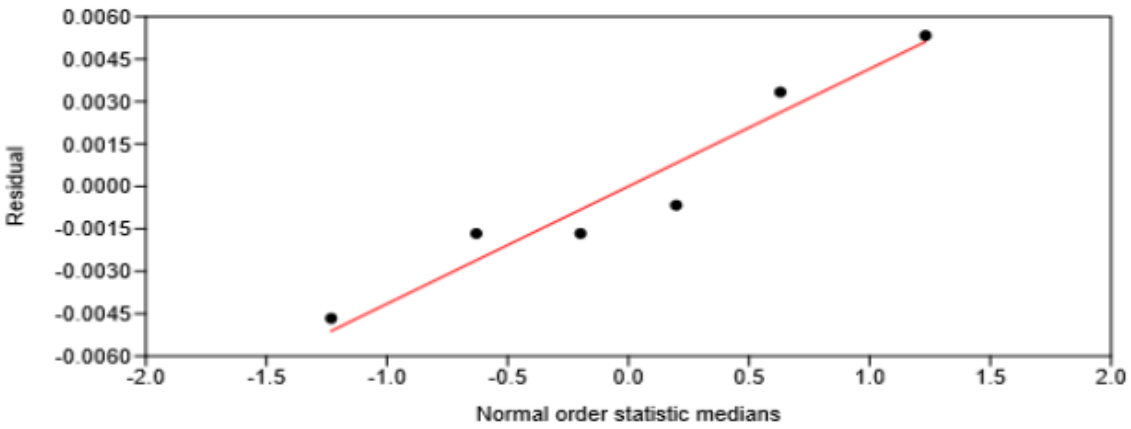
At 15 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.9868	0.75
p(normal)	0.7804	0
Anderson-Darling A	0.2042	0.4878
p(normal)	0.5652	0.05651
p(Monte Carlo)	0.7758	0.0001
Lilliefors L	0.2194	0.3848
p(normal)	0.8367	0.08879
p(Monte Carlo)	0.781	0.0001
Jarque-Bera JB	0.3098	0.5312
p(normal)	0.8565	0.7667
p(Monte Carlo)	0.7817	0.0001

Residuals

Shapiro-Wilk W	0.9319
p(normal)	0.5952



At 15 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	0.1226667	Mean:	0.3706667		
95% conf.:	(4.0438 5.2362)	95% conf.:	(30.623 31.617)		
Variance:	0.0576	Variance:	4.00E-02		
Difference between means:	0.248				
95% conf. interval (parametric):	(25.979 26.981)				
95% conf. interval (bootstrap):	(26.187 26.773)				
t :	146.81	p (same mean):	1.29E-08	Critical t value (p	2.7764
Uneq. var. t :	146.81	p (same mean):	2.11E-08	Critical t value (p	2.8114
Monte Carlo permutation:	p (same mean):	0.1048			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	7.46E+04	Cohen´s D:	119.9		
Decisive evidence for unequal means	Huge effect size				

At 20 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	0.143	0.486
Max	0.145	0.49
Sum	0.432	1.462
Mean	0.144	0.4873333
Std. error	0.00057735	0.001333333
Variance	1.00E-06	5.33E-06
Stand. dev	0.001	0.002309401
Median	0.144	0.486
25 prcntil	0.143	0.486
75 prcntil	0.145	0.49
Mode	NA	0.486
Skewness	0	1.73E+00
Kurtosis	-2.333333	-2.333333
Geom. mean	0.1439977	0.4873297
Harm. mean	0.1439954	0.4873261
Coeff. var	0.6944444	0.4738853

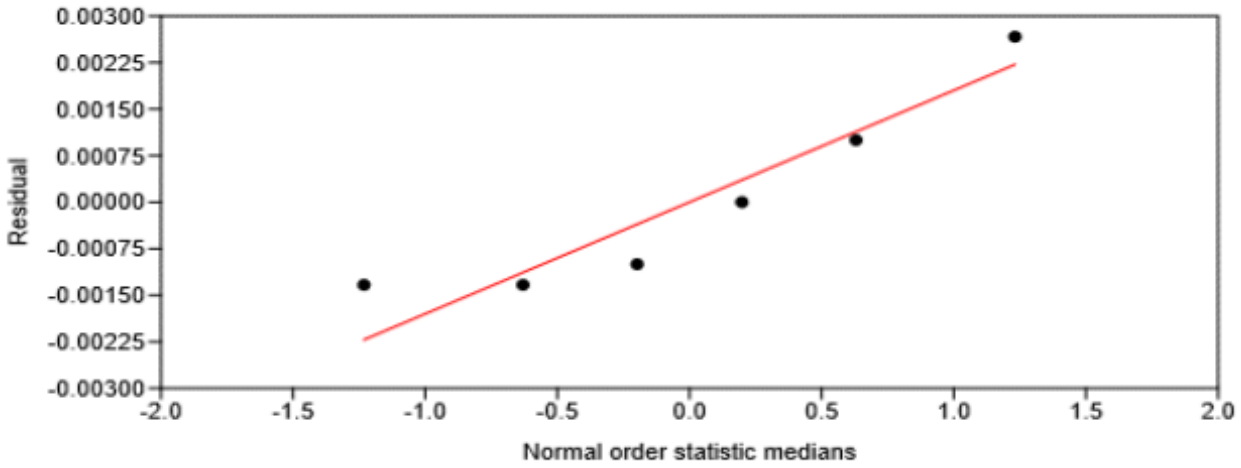
At 20 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	1	0.75
p(normal)	1	0
Anderson-Darling A	0.1895	0.4878
p(normal)	0.6307	0.05651
p(Monte Carlo)	0.9999	0.0001
Lilliefors L	0.1747	0.3848
p(normal)	1	0.08879
p(Monte Carlo)	1	0.0001
Jarque-Bera JB	0.2812	0.5312
p(normal)	0.8688	0.7667
p(Monte Carlo)	1	0.0001

Residuals

Shapiro-Wilk W	0.8804
p(normal)	0.2709



At 20 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	0.144	Mean:	0.4873333		
95% conf.:	(2.1132 3.1135)	95% conf.:	(18.239 21.195)		
Variance:	0.040533	Variance:	3.54E-01		
Difference between means:	0.3433333				
95% conf. interval (parametric):	(16.096 18.11)				
95% conf. interval (bootstrap):	(16.51 17.687)				
t :	47.161	p (same mean):	1.21E-06	Critical t value (p	2.7764
Uneq. var. t :	47.161	p (same mean):	0.00010899	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.106			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	2979	Cohen´s D:	38.51		
Decisive evidence for unequal means	Huge effect size				

At 25 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	0.2	0.603
Max	0.201	0.61
Sum	0.602	1.821
Mean	0.2006667	0.607
Std. error	0.000333333	0.002081666
Variance	3.33E-07	1.30E-05
Stand. dev	0.00057735	0.003605551
Median	0.201	0.608
25 prcntil	0.2	0.603
75 prcntil	0.201	0.61
Mode	0.201	NA
Skewness	-1.732051	-1.15E+00
Kurtosis	-2.333333	-2.333333
Geom. mean	0.2006661	0.6069929
Harm. mean	0.2006656	0.6069857
Coeff. var	0.2877161	0.5939953

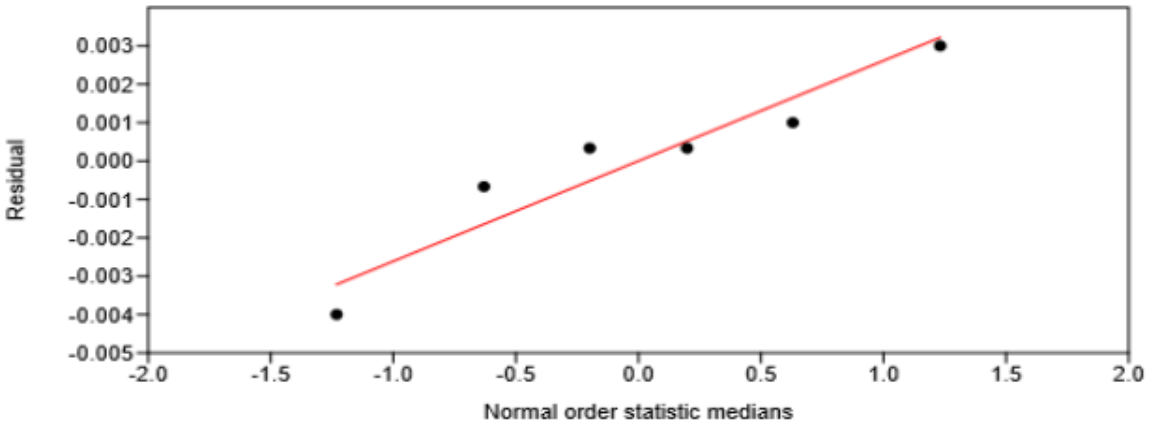
At 25 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.75	0.9423
p(normal)	0	0.5367
Anderson-Darling A	0.4878	0.2547
p(normal)	0.05651	0.399
p(Monte Carlo)	0.0001	0.5426
Lilliefors L	0.3848	0.2759
p(normal)	0.08879	0.5156
p(Monte Carlo)	0.0001	0.5328
Jarque-Bera JB	0.5313	0.3919
p(normal)	0.7667	0.8221
p(Monte Carlo)	0.0001	0.5342

Residuals

Shapiro-Wilk W	0.9258
p(normal)	0.5479



At 25 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	0.2006667	Mean:	0.607		
95% conf.:	(6.2902 7.8965)	95% conf.:	(48.532 49.128)		
Variance:	0.10453	Variance:	1.44E-02		
Difference between means:	0.4063333				
95% conf. interval (parametric):	(41.184 42.289)				
95% conf. interval (bootstrap):	(41.443 42.07)				
t :	209.62	p (same mean):	3.11E-09	Critical t value (p	2.7764
Uneq. var. t :	209.62	p (same mean):	1.88E-06	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.098			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	1.81E+05	Cohen´s D:	171.2		
Decisive evidence for unequal means		Huge effect size			

At 30 ppm

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	0.246	0.67
Max	0.248	0.672
Sum	0.741	2.013
Mean	0.247	0.671
Std. error	0.00057735	0.00057735
Variance	1.00E-06	1.00E-06
Stand. dev	0.001	0.001
Median	0.247	0.671
25 prcntil	0.246	0.67
75 prcntil	0.248	0.672
Mode	NA	NA
Skewness	0	-9.99E-13
Kurtosis	-2.333333	-2.333333
Geom. mean	0.2469987	0.6709995
Harm. mean	0.2469973	0.670999
Coeff. var	0.4048583	0.1490313

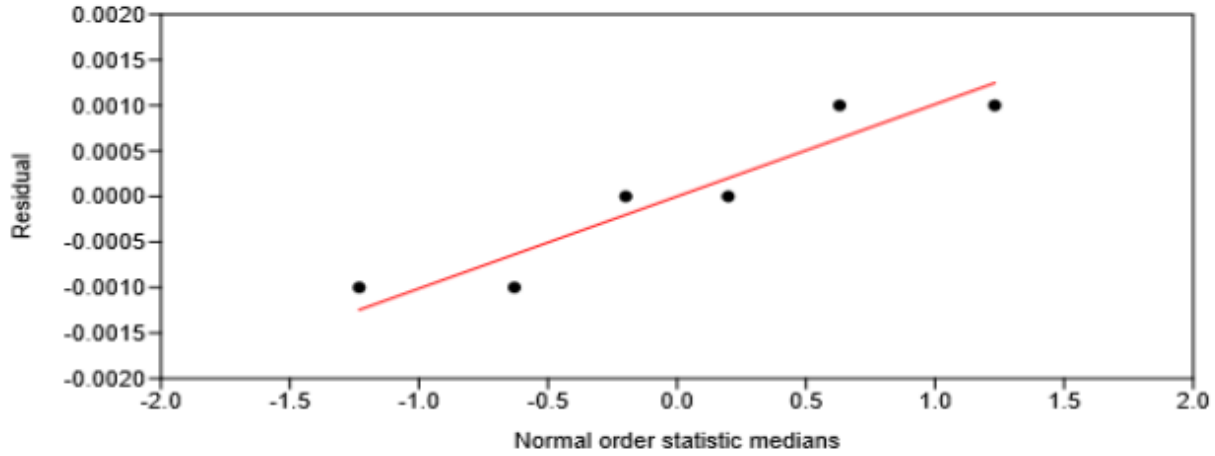
At 30 ppm

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	1	1
p(normal)	1	1
Anderson-Darling A	0.1895	0.1895
p(normal)	0.6307	0.6307
p(Monte Carlo)	1	0.9999
Lilliefors L	0.1747	0.1747
p(normal)	1	1
p(Monte Carlo)	1	1
Jarque-Bera JB	0.2812	0.2813
p(normal)	0.8688	0.8688
p(Monte Carlo)	1	1

Residuals

Shapiro-Wilk W	0.8532
p(normal)	0.167



At 30 ppm

Group Comparison Test

t tests for equal means

VG EtAc Fraction		Ascorbic acid			
N:	3	N:	3		
Mean:	0.247	Mean:	0.671		
95% conf.:	(0.24452 0.24948)	95% conf.:	(0.66852 0.67348)		
Variance:	1.00E-06	Variance:	1.00E-06		
Difference between means:	0.424				
95% conf. interval (parametric):	(0.42173 0.42627)				
95% conf. interval (bootstrap):	(0.42267 0.42533)				
t :	519.29	p (same mean):	8.25E-11	Critical t value (p	2.7764
Uneq. var. t :	519.29	p (same mean):	8.25E-11	Critical t value (p	2.7764
Monte Carlo permutation:	p (same mean):	0.0342			
Exact permutation:	p (same mean):	0.095238			
Bayes factor:	8.62E+05	Cohen´s D:	424		
Decisive evidence for unequal means	Huge effect size				

IC50

Descriptives

	VG EtAc Fraction	Ascorbic acid
N	3	3
Min	56.57	20.43
Max	63.16	21.2
Sum	179.74	62.15
Mean	59.91333	20.71667
Std. error	1.902983	0.2430592
Variance	1.09E+01	1.77E-01
Stand. dev	3.296063	0.4209909
Median	60.01	20.52
25 prcntil	56.57	20.43
75 prcntil	63.16	21.2
Mode	NA	NA
Skewness	-0.1318621	1.64E+00
Kurtosis	-2.333333	-2.333333
Geom. mean	59.85273	20.71384
Harm. mean	59.79203	20.71103
Coeff. var	5.501385	2.032136

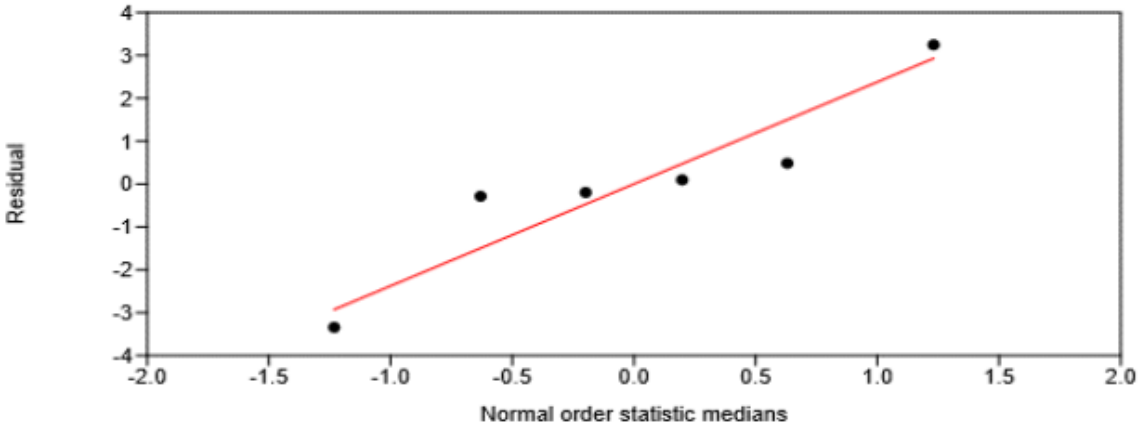
IC50

Test for Normality

	VG EtAc Fraction	Ascorbic acid
N	3	3
Shapiro-Wilk W	0.9994	0.8363
p(normal)	0.9515	0.2045
Anderson-Darling A	0.1902	0.3799
p(normal)	0.6273	0.1396
p(Monte Carlo)	0.9511	0.2051
Lilliefors L	0.1784	0.3465
p(normal)	1	0.1848
p(Monte Carlo)	0.9524	0.1996
Jarque-Bera JB	0.2827	0.5063
p(normal)	0.8682	0.7763
p(Monte Carlo)	0.9501	0.2025

Residuals

Shapiro-Wilk W	0.8532
p(normal)	0.167



IC50

Group Comparison Test

t tests for equal means

VG EtAc Fraction	Ascorbic acid				
N:	3	N:	3		
Mean:	59.913	Mean:	20.717		
95% conf.:	(51.725 68.101)	95% conf.:	(19.671 21.762)		
Variance:	1.09E+01	Variance:	1.77E-01		
Difference between means:	39.197				
95% conf. interval (parametric):	(33.87 44.523)				
95% conf. interval (bootstrap):	(35.98 42.343)				
t :	20.432	p (same mean):	3.39E-05	Critical t value (p	2.7764
Uneq. var. t :	20.432	p (same mean):	2.05E-03	Critical t value (p	4.3027
Monte Carlo permutation:	p (same mean):	0.0999			
Exact permutation:	p (same mean):	0.14286			
Bayes factor:	2.55E+02	Cohen´s D:	16.68		
Decisive evidence for unequal means	Huge effect size				

Raw Data for Acetylcholinesterase inhibition of the ethyl acetate fraction of Voacanga globosa against Galantamine

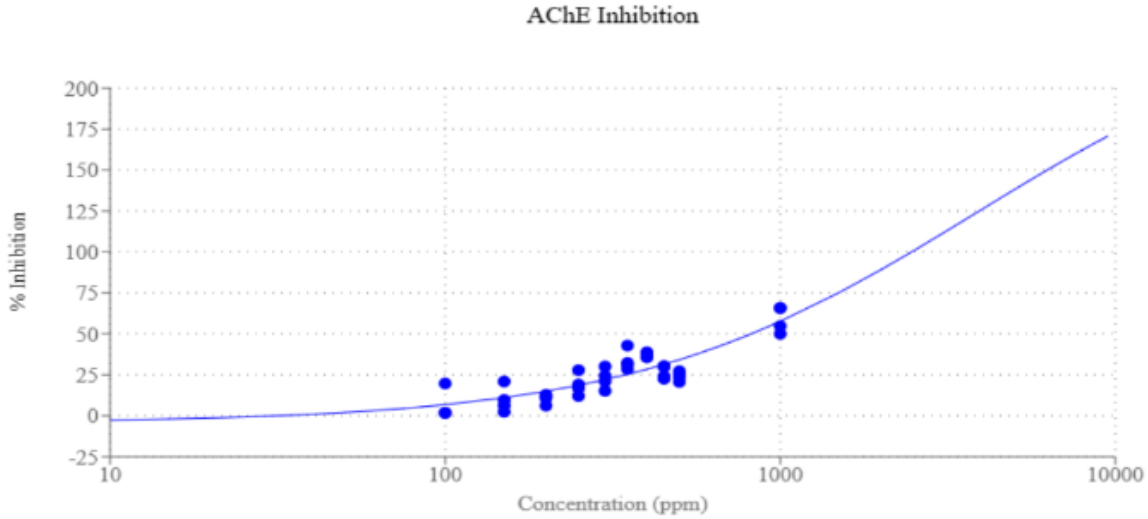
Acetylcholinesterase Inhibition of VGE

Group	% Inhibition (Mean ± SD)	p value
Galantamine	99.59 ± 0.14	p < 0.05 (There is a SIGNIFICANT difference.)
Sample 1000 ppm	68.39 ± 7.98 *	
Sample 500 ppm	33.51 ± 2.81 *	
Sample 450 ppm	36.04 ± 3.98 *	
Sample 400 ppm	46.41 ± 1.45 *	
Sample 350 ppm	42.99 ± 6.29 *	
Sample 300 ppm	32.00 ± 6.22 *	
Sample 250 ppm	28.25 ± 6.61 *	
Sample 150 ppm	19.11 ± 7.98 *	
Sample 100 ppm	15.12 ± 9.29 *	

**vs Galantamine (positive control), p<0.05 (ANOVA with Tukey's HSD Test.)*

	POSITIVE	SAMPLE									
Trial	Galantamine	Sample 1000 ppm	Sample 500 ppm	Sample 450 ppm	Sample 400 ppm	Sample 350 ppm	Sample 300 ppm	Sample 250 ppm	Sample 200 ppm	Sample 150 ppm	Sample 100 ppm
1	99.39	64.05	32.76	39.25	48.01	52.14	39.40	37.14	22.08	30.22	29.00
2	99.63	59.33	34.79	31.87	45.47	41.55	33.69	26.11	21.35	18.96	11.04
3	99.61	75.09	36.50	39.62	47.22	40.43	24.54	21.36	15.46	15.60	9.30
4	99.73	75.09	29.98	33.41	44.94	37.86	30.36	28.39	20.12	11.67	11.13
MEAN	**99.59**	**68.39**	**33.51**	**36.04**	**46.41**	**42.99**	**32.00**	**28.25**	**19.75**	**19.11**	**15.12**
SD	**0.14**	**7.98**	**2.81**	**3.98**	**1.45**	**6.29**	**6.22**	**6.61**	**2.97**	**7.98**	**9.29**
p-value (vs POSI)	NA	4.792E-07	5.751E-14	6.151E-14	9.627E-13	2.2E-13	5.629E-14	5.53E-14	5.51E-14	5.51E-14	5.51E-14
Interpretation	NA	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.

IC50 for Acetylcholinesterase Inhibition



IC_{50} Regression Results [Sample]

Parameter	Value
IC_{50}	3817.1257
Equations	(show alternative)
Equation	$Y = -4.23 + \frac{251.2132 + 4.23}{1 + (\frac{X}{3817.1257})^{-0.8515}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{IC50})^{Hill\ coefficient}}$

Acetylcholinesterase Inhibition

Group	Concentration	% INH
Galantamine		99.39
Galantamine		99.63
Galantamine		99.61
Galantamine		99.73
Sample 1000 ppm	1000	64.05
Sample 1000 ppm	1000	59.33
Sample 1000 ppm	1000	75.09
Sample 1000 ppm	1000	75.09
Sample 500 ppm	500	32.76
Sample 500 ppm	500	34.79
Sample 500 ppm	500	36.50
Sample 500 ppm	500	29.98
Sample 450 ppm	450	39.25
Sample 450 ppm	450	31.87
Sample 450 ppm	450	39.62
Sample 450 ppm	450	33.41
Sample 400 ppm	400	48.01
Sample 400 ppm	400	45.47
Sample 400 ppm	400	47.22
Sample 400 ppm	400	44.94
Sample 350 ppm	350	52.14
Sample 350 ppm	350	41.55
Sample 350 ppm	350	40.43
Sample 350 ppm	350	37.86
Sample 300 ppm	300	39.40
Sample 300 ppm	300	33.69
Sample 300 ppm	300	24.54
Sample 300 ppm	300	30.36
Sample 250 ppm	250	37.14
Sample 250 ppm	250	26.11
Sample 250 ppm	250	21.36
Sample 250 ppm	250	28.39
Sample 200 ppm	200	22.08
Sample 200 ppm	200	21.35
Sample 200 ppm	200	15.46
Sample 200 ppm	200	20.12
Sample 150 ppm	150	30.22
Sample 150 ppm	150	18.96
Sample 150 ppm	150	15.60
Sample 150 ppm	150	11.67
Sample 100 ppm	100	29.00
Sample 100 ppm	100	11.04
Sample 100 ppm	100	9.30
Sample 100 ppm	100	11.13

Descriptives

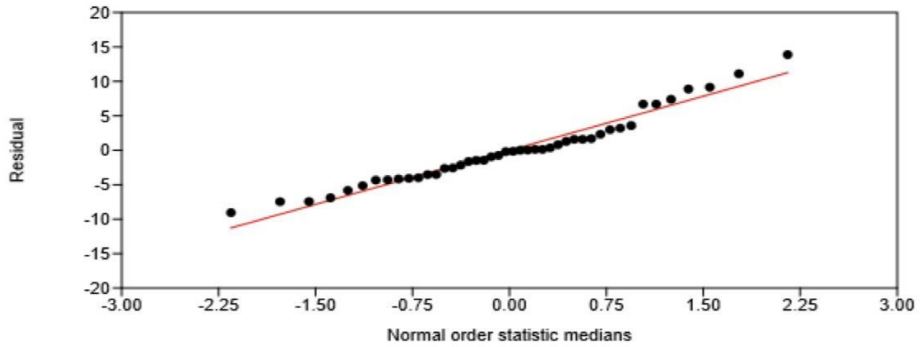
	Galantamine	Sample 1000 ppm	Sample 500 ppm	Sample 450 ppm	Sample 400 ppm	Sample 350 ppm	Sample 300 ppm	Sample 250 ppm	Sample 200 ppm	Sample 150 ppm	Sample 100 ppm
N	4	4	4	4	4	4	4	4	4	4	4
Min	99.39	59.33	29.98	31.87	44.94	37.86	24.54	21.36	15.46	11.67	9.3
Max	99.73	75.09	36.5	39.62	48.01	52.14	39.4	37.14	22.08	30.22	29
Sum	398.36	273.56	134.03	144.15	185.64	171.98	127.99	113	79.01	76.45	60.47
Mean	99.59	68.39	33.5075	36.0375	46.41	42.995	31.9975	28.25	19.7525	19.1125	15.1175
Std. error	0.071647	3.986427	1.402435	1.988011	0.7223	3.144658	3.108554	3.305342	1.486884	3.990909	4.646623
Variance	0.020533	63.5664	7.867292	15.80876	2.086867	39.5555	38.65242	43.70113	8.843292	63.70942	86.36442
Stand. dev	0.143295	7.972854	2.804869	3.976023	1.444599	6.289316	6.217107	6.610683	2.973767	7.981818	9.293246
Median	99.62	69.57	33.775	36.33	46.345	40.99	32.025	27.25	20.735	17.28	11.085
25 prcntil	99.445	60.51	30.675	32.255	45.0725	38.5025	25.995	22.5475	16.625	12.6525	9.735
75 prcntil	99.705	75.09	36.0725	39.5275	47.8125	49.4925	37.9725	34.9525	21.8975	27.405	24.5325
Mode	NA	75.09	NA	NA	NA	NA	NA	NA	NA	NA	NA
Skewness	-1.174585	-0.294522	-0.46537	-0.12079	0.137181	1.633355	-0.024001	0.843942	-1.580775	1.19933	1.949824
Kurtosis	2.208383	-4.324398	-0.680008	-5.22343	-3.982764	2.983216	0.134578	1.37188	2.500021	1.645463	3.847295
Geom. mean	99.58992	68.0361	33.41802	35.8718	46.39316	42.67334	31.53504	27.69197	19.56855	17.97141	13.49231
Harm. mean	99.58985	67.67916	33.32729	35.70596	46.37634	42.3757	31.06772	27.16196	19.36923	16.97608	12.40554
Coeff. var	0.143885	11.65792	8.37087	11.03302	3.112689	14.62802	19.42998	23.40065	15.05514	41.76229	61.47343

Test for Normality

	Galantamine	Sample 1000 ppm	Sample 500 ppm	Sample 450 ppm	Sample 400 ppm	Sample 350 ppm	Sample 300 ppm	Sample 250 ppm	Sample 200 ppm	Sample 150 ppm	Sample 100 ppm
N	4	4	4	4	4	4	4	4	4	4	4
Shapiro-Wilk W	0.9118	0.8415	0.9838	0.8363	0.9208	0.8428	0.9997	0.961	0.852	0.9266	0.7091
p(normal)	0.492	0.1997	0.9238	0.1847	0.5414	0.2036	0.9992	0.7849	0.2327	0.5744	0.01473
Anderson-Darling A	0.3198	0.3861	0.1738	0.3962	0.263	0.4265	0.1581	0.227	0.3922	0.274	0.6755
p(normal)	0.3171	0.1942	0.8046	0.1797	0.4746	0.1424	0.8612	0.5782	0.1853	0.4394	0.02208
p(Monte Carlo)	0.3977	0.223	0.9302	0.2061	0.6009	0.168	0.9835	0.7237	0.2125	0.5517	0.0145
Lilliefors L	0.3055	0.2996	0.1763	0.2904	0.2424	0.3409	0.1461	0.2416	0.2992	0.2576	0.4161
p(normal)	0.219	0.2435	0.9553	0.2858	0.5688	0.1073	1	0.5743	0.2456	0.4701	0.01681
p(Monte Carlo)	0.1904	0.2169	0.9302	0.2643	0.6222	0.1064	0.9884	0.6305	0.2282	0.5096	0.0082
Jarque-Bera JB	0.4433	0.5453	0.3258	0.6027	0.5036	0.7001	0.233	0.3307	0.6805	0.4799	0.9235
p(normal)	0.8012	0.7614	0.8497	0.7398	0.7774	0.7046	0.89	0.8476	0.7116	0.7867	0.6302
p(Monte Carlo)	0.5885	0.3193	0.8022	0.2396	0.4131	0.1306	0.9248	0.7959	0.138	0.4801	0.0151

Residuals

Shapiro-Wilk W	0.9571
p(normal)	0.1015



Test for Homogeneity of Variance

Levene´s test for homogeneity of variance, from means	p (same):	0.03318
Levene´s test, from medians	p (same):	0.448

Group Comparison Test

ONE-WAY ANOVA

Test for equal means

	Sum of sqrs	df	Mean squa	F	p (same)	Critical F (p=0.05)
Between groups:	24529	10	2452.9	72.89	**1.46E-19**	2.133
Within groups:	1110.53	33	33.6524	Permutation p (n=99999)		
Total:	25639.5	43	1.00E-05			

Components of variance (only for random effects):

Var(group): 604.811 Var(error): 33.6524 ICC: 0.947292

omega2: 0.9423

Levene´s test for homogeneity of variance, from means	p (same):	3.32E-02
Levene´s test, from medians	p (same):	0.448

Welch F test in the case of unequal variances: F=892.2, df=12.04, p=1.098E-15

Bayes factor: 4.062E16 (decisive evidence for unequal means)

Post Hoc Analysis

Tukey's HSD Test

**Tukey's Q below diagonal, p-value above diagonal. Significant difference highlighted in pink (p<0.05)*

	Galantamine	Sample 1000 ppm	Sample 500 ppm	Sample 450 ppm	Sample 400 ppm	Sample 350 ppm	Sample 300 ppm	Sample 250 ppm	Sample 200 ppm	Sample 150 ppm	Sample 100 ppm
Galantamine		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sample 1000 ppm	10.76		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sample 500 ppm	22.78	12.03		1.00	0.10	0.45	1.00	0.97	0.06	0.04	0.00
Sample 450 ppm	21.91	11.15	0.87		0.33	0.83	0.99	0.71	0.01	0.01	0.00
Sample 400 ppm	18.33	7.58	4.45	3.58		1.00	0.04	0.00	0.00	0.00	0.00
Sample 350 ppm	19.51	8.76	3.27	2.40	1.18		0.25	0.04	0.00	0.00	0.00
Sample 300 ppm	23.30	12.55	0.52	1.39	4.97	3.79		1.00	0.14	0.10	0.01
Sample 250 ppm	24.60	13.84	1.81	2.69	6.26	5.08	1.29		0.60	0.50	0.09
Sample 200 ppm	27.53	16.77	4.74	5.61	9.19	8.01	4.22	2.93		1.00	0.99
Sample 150 ppm	27.75	16.99	4.96	5.84	9.41	8.23	4.44	3.15	0.22		1.00
Sample 100 ppm	29.12	18.37	6.34	7.21	10.79	9.61	5.82	4.53	1.60	1.38	

Raw Data

Absorbance readings of the positive, negative controls, and samples

Reading	Avg. time	Negative Control				Positive				1000				500			
		Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2
1	0.00	0.2416	0.2289	0.2383	0.2382	0.1483	0.1588	0.1543	0.1536	3.2330	3.1891	3.5816	3.5816	1.8275	2.2725	2.399	2.154
2	0.42	0.2809	0.2751	0.2987	0.3097	0.1513	0.1569	0.1535	0.1536	3.2583	3.3191	3.4787	3.4787	2.0107	2.2091	2.3701	2.0916
3	0.83	0.3298	0.3197	0.3537	0.3666	0.1515	0.1577	0.1543	0.1539	3.2983	3.3468	3.5011	3.5011	2.0286	2.1953	2.3911	2.1016
4	1.25	0.3763	0.3621	0.4153	0.4228	0.1513	0.1583	0.1551	0.154	3.303	3.3945	3.5213	3.5213	2.0585	2.217	2.4431	2.1469
5	1.67	0.4239	0.4049	0.4821	0.4717	0.1518	0.1589	0.1543	0.1542	3.3296	3.4223	3.5303	3.5303	2.1009	2.2613	2.4951	2.204
6	2.08	0.4795	0.4494	0.5466	0.5236	0.1529	0.1586	0.1545	0.154	3.3455	3.4505	3.5188	3.5188	2.1521	2.3221	2.5374	2.2587
7	2.50	0.5362	0.4941	0.6084	0.5788	0.1536	0.1586	0.1548	0.154	3.4296	3.4551	3.6477	3.6477	2.1881	2.3805	2.5863	2.3125
8	2.92	0.5943	0.538	0.669	0.633	0.1541	0.1587	0.1548	0.1543	3.4406	3.5139	3.6532	3.6532	2.2211	2.4271	2.6303	2.3678
9	3.33	0.6515	0.5812	0.7298	0.6878	0.1548	0.1589	0.1549	0.1542	3.4224	3.5349	3.6445	3.6445	2.253	2.4624	2.6694	2.4031
10	3.75	0.7069	0.6242	0.7895	0.7405	0.1546	0.159	0.1544	0.1546	3.419	3.5589	3.6642	3.6642	2.2987	2.4991	2.6968	2.4316
11	4.17	0.7622	0.6662	0.8464	0.7922	0.1547	0.1591	0.155	0.1548	3.46	3.5627	3.6511	3.6511	2.3295	2.5363	2.7254	2.4633
12	4.58	0.8156	0.7088	0.9007	0.8422	0.1555	0.1594	0.1555	0.1554	3.5246	3.5855	3.6977	3.6977	2.3586	2.5591	2.7502	2.5028
13	5.00	0.8668	0.7496	0.9537	0.8907	0.1553	0.1594	0.1557	0.1556	3.5085	3.6356	3.7041	3.7041	2.3828	2.5893	2.7752	2.538
14	5.42	0.917	0.7908	1.0058	0.9387	0.1559	0.1595	0.1566	0.1557	3.5059	3.6031	3.7576	3.7576	2.4144	2.6158	2.805	2.5637
15	5.83	0.9656	0.8306	1.0559	0.9852	0.1556	0.1596	0.1564	0.1553	3.5441	3.6054	3.7106	3.7106	2.442	2.6403	2.8253	2.5897
16	6.25	1.0155	0.8694	1.1037	1.0303	0.1562	0.1602	0.1574	0.156	3.5312	3.6851	3.7936	3.7936	2.4687	2.6712	2.8468	2.6154
17	6.67	1.065	0.9071	1.1511	1.074	0.1560	0.1601	0.1575	0.1558	3.5726	3.6378	3.7913	3.7913	2.5012	2.7017	2.8802	2.6516
18	7.08	1.1136	0.9441	1.1964	1.1172	0.1567	0.1605	0.1576	0.156	3.5728	3.6565	3.7826	3.7826	2.5208	2.7278	2.9058	2.675
19	7.50	1.1617	0.9795	1.2392	1.1586	0.1565	0.1605	0.1577	0.1562	3.5544	3.6796	3.7462	3.7462	2.5477	2.7454	2.9349	2.6981
20	7.92	1.2065	1.0136	1.28	1.1987	0.1566	0.1607	0.1581	0.1559	3.6127	3.6935	3.751	3.751	2.5705	2.7701	2.9609	2.7229
21	8.33	1.2525	1.0468	1.318	1.2375	0.1572	0.161	0.1582	0.1564	3.6068	3.6783	3.7531	3.7531	2.6017	2.7972	2.9867	2.7524
22	8.75	1.2925	1.0787	1.3551	1.2739	0.1574	0.161	0.1581	0.156	3.6459	3.7737	3.793	3.793	2.6268	2.8257	3.0205	2.7694
23	9.17	1.33	1.1092	1.3899	1.3083	0.1572	0.161	0.158	0.1562	3.6559	3.7812	3.8095	3.8095	2.6498	2.8392	3.0216	2.8036
24	9.58	1.3656	1.1404	1.4234	1.3423	0.1577	0.1617	0.1584	0.1567	3.5844	3.7603	3.7631	3.7631	2.668	2.86	3.0568	2.8311
25	10.00	1.3985	1.168	1.4547	1.3727	0.1577	0.1616	0.1578	0.1564	3.6561	3.7903	3.8122	3.8122	2.6943	2.8829	3.0726	2.8541
26	10.42	1.4275	1.1962	1.4842	1.4025	0.1576	0.1617	0.1581	0.1564	3.6736	3.7815	3.8157	3.8157	2.7182	2.9148	3.0934	2.8703
27	10.83	1.4562	1.2234	1.5109	1.4307	0.1579	0.1618	0.1583	0.1567	3.6727	3.7589	3.7832	3.7832	2.7306	2.9293	3.0995	2.9
28	11.25	1.4839	1.2478	1.5368	1.4565	0.1576	0.1622	0.158	0.1567	3.7351	3.7444	3.7963	3.7963	2.7589	2.9527	3.1302	2.9162
29	11.67	1.5079	1.2729	1.562	1.4812	0.1581	0.1622	0.1585	0.1568	3.6678	3.8578	3.8147	3.8147	2.7687	2.9787	3.144	2.9385
30	12.08	1.534	1.2954	1.5834	1.5038	0.1582	0.1625	0.1577	0.1567	3.7281	3.8319	3.8047	3.8047	2.7941	2.9923	3.1651	2.9599
31	12.50	1.5552	1.3185	1.6032	1.5238	0.1586	0.1626	0.1582	0.157	3.7212	3.8067	3.83	3.83	2.8017	3.0147	3.1711	2.9862

slope	0.11059	0.088666	0.111476	0.104031	0.000627	0.000389	0.000408	0.000283	0.037273	0.042174	0.025825	0.025825	0.069723	0.067616	0.065839	0.072604
slope trial	0.099628		0.10775389		0.000508161		0.000345484		0.039723358		0.025824605		0.068669299		0.069221781	
ave slope	0.1037				0.0004				0.0328				0.0689			
%inh	-6.65302	14.48966	-7.50838	-0.32826	99.39485	99.625	99.60624	99.72738	64.05396	59.32728	75.09464	75.09464	32.759	34.79106	36.50422	29.98021
ave %inh	0.0000				99.5884				68.3926				33.5086			
sd	10.1767				0.1396				7.9758				2.8065			

450				400				350				300			
Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2
1.4985	1.8986	2.2028	2.238	1.141	1.2229	1.3181	1.1996	0.9623	0.8695	1.0180	1.0158	1.0156	1.2637	1.1548	1.1853
1.4279	2.0169	2.1657	2.1979	1.1484	1.323	1.2682	1.195	0.9817	0.9324	1.031	1.0091	1.0157	1.1804	1.1728	1.2096
1.4751	2.016	2.1768	2.2122	1.1643	1.3563	1.2716	1.2177	1.0249	0.9598	1.0557	1.0293	1.0502	1.1847	1.2121	1.1192
1.5167	2.0483	2.1918	2.258	1.1878	1.3731	1.3013	1.236	1.0386	0.9883	1.0838	1.0654	1.0909	1.2127	1.2491	1.1565
1.5427	2.0809	2.2336	2.3071	1.218	1.4065	1.3392	1.2675	1.0663	1.0284	1.1164	1.0839	1.1131	1.2502	1.2865	1.2105
1.5915	2.1143	2.2822	2.3576	1.245	1.4313	1.3681	1.3076	1.0913	1.0651	1.1519	1.1215	1.1485	1.2928	1.3312	1.25
1.6164	2.1642	2.3212	2.3986	1.2797	1.4613	1.399	1.3552	1.1118	1.0999	1.1782	1.1496	1.1786	1.3445	1.3724	1.2907
1.6516	2.2009	2.3552	2.4406	1.3133	1.489	1.4243	1.3913	1.1389	1.1348	1.2085	1.1883	1.2067	1.3893	1.4144	1.3311
1.6773	2.2395	2.3931	2.479	1.3413	1.5168	1.453	1.4261	1.1742	1.1599	1.2408	1.2209	1.2442	1.4269	1.4579	1.3688
1.7117	2.2736	2.4267	2.5132	1.365	1.5408	1.4781	1.4532	1.1873	1.1909	1.2665	1.2531	1.2728	1.4622	1.4967	1.4053
1.7321	2.3124	2.4547	2.5487	1.3868	1.5678	1.5044	1.4818	1.2186	1.2147	1.2976	1.2853	1.3005	1.4924	1.5342	1.4391
1.7628	2.3402	2.4784	2.5789	1.413	1.5914	1.5269	1.5091	1.2213	1.2406	1.3242	1.3187	1.3285	1.5215	1.5714	1.4746
1.7893	2.3723	2.5082	2.6082	1.435	1.6139	1.5512	1.5317	1.2599	1.2648	1.3542	1.3492	1.3577	1.5506	1.6083	1.5067
1.82	2.4038	2.5332	2.6355	1.4577	1.6383	1.5749	1.5621	1.2586	1.2905	1.3823	1.3814	1.3839	1.5797	1.6419	1.539
1.8419	2.4306	2.5584	2.667	1.4783	1.6621	1.5971	1.5778	1.2875	1.3151	1.4082	1.4045	1.4125	1.6081	1.6733	1.5722
1.8693	2.4594	2.5877	2.69	1.5016	1.6853	1.6221	1.5981	1.3229	1.3391	1.4335	1.4294	1.4359	1.6363	1.7069	1.6067
1.897	2.486	2.6111	2.7222	1.5233	1.7076	1.643	1.6192	1.3178	1.3619	1.4593	1.4524	1.4621	1.6633	1.738	1.636
1.9218	2.5129	2.6316	2.7454	1.5439	1.7293	1.6653	1.642	1.3636	1.3873	1.4849	1.4793	1.4895	1.6921	1.7693	1.6664
1.9434	2.538	2.6550	2.7715	1.566	1.7519	1.6877	1.6619	1.3604	1.4084	1.5094	1.5024	1.5148	1.7184	1.7994	1.6992
1.9711	2.5675	2.681	2.7964	1.5863	1.7754	1.7097	1.6835	1.4041	1.4317	1.534	1.5272	1.5373	1.7453	1.8287	1.7235
1.9963	2.5957	2.7058	2.8302	1.6053	1.7957	1.732	1.7055	1.4016	1.4551	1.5585	1.5518	1.5625	1.7717	1.8567	1.7534
2.0216	2.6178	2.7242	2.8368	1.6251	1.8156	1.7539	1.7234	1.4458	1.4773	1.5819	1.5772	1.5861	1.7983	1.8871	1.7795
2.0478	2.6417	2.7466	2.87	1.6452	1.8367	1.7747	1.7427	1.4382	1.4984	1.6032	1.6021	1.608	1.8238	1.9137	1.8054
2.072	2.6726	2.7717	2.8878	1.664	1.8563	1.7948	1.7609	1.4848	1.5216	1.625	1.6246	1.6326	1.8483	1.9406	1.8292
2.0886	2.6938	2.798	2.921	1.6836	1.8764	1.8156	1.7793	1.4739	1.5415	1.6472	1.6474	1.6503	1.8722	1.9657	1.8561
2.1146	2.7117	2.8149	2.9379	1.7048	1.8975	1.836	1.7987	1.5207	1.5634	1.6681	1.672	1.6758	1.8975	1.9924	1.8815
2.1422	2.7381	2.8388	2.9693	1.723	1.9167	1.8546	1.8169	1.5088	1.5827	1.6893	1.6948	1.696	1.9193	2.0169	1.9066
2.1561	2.758	2.8555	2.9774	1.7423	1.9345	1.8734	1.8344	1.5551	1.6028	1.7095	1.7166	1.7177	1.9435	2.0415	1.9264
2.1767	2.7847	2.8809	3.0009	1.7622	1.9536	1.8922	1.85	1.5467	1.6222	1.7284	1.7361	1.7386	1.9649	2.0626	1.9507
2.1958	2.7959	2.9039	3.0128	1.7762	1.9728	1.9112	1.8656	1.5544	1.6414	1.7476	1.7572	1.757	1.986	2.085	1.9742
2.2169	2.8225	2.9107	3.0361	1.7949	1.988	1.9274	1.8818	1.5964	1.659	1.7668	1.7776	1.778	2.008	2.1049	1.9887

0.062987	0.07064	0.062607	0.069052	0.053905	0.056544	0.054724	0.057092	0.049631	0.060608	0.061771	0.064434	0.062832	0.068761	0.078243	0.072207
0.066813409		0.065829263		0.055224744		0.055908358		0.055119501		0.063102166		0.065796602		0.075224835	
0.0663				0.0556				0.0591				0.0705			
39.25483	31.87489	39.6217	33.40624	48.01352	45.46852	47.22367	44.9398	52.13569	41.54933	40.42816	37.85983	39.40481	33.68613	24.54237	30.36332
36.0394				46.4114				42.9933				31.9992			
3.9770				1.4470				6.2876				6.2174			

250				200				150				100			
Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2	Trial 1, Replicate 1	Trial 1, Replicate 2	Trial 2, Replicate 1	Trial 2, Replicate 2
0.8978	1.0164	0.9309	1.2373	0.7432	0.7514	0.826	0.7587	0.5873	0.6628	0.7063	0.6294	0.4419	0.5083	0.4908	0.5286
0.8454	0.9076	0.9784	1.1828	0.7507	0.8092	0.8293	0.7625	0.5723	0.6724	0.7168	0.6569	0.4937	0.5402	0.5218	0.5535
0.8807	0.9357	1.0177	1.0249	0.7799	0.8319	0.875	0.8001	0.6096	0.714	0.755	0.7062	0.5643	0.589	0.5692	0.5978
0.9106	0.9877	1.0631	1.0389	0.8225	0.8803	0.9255	0.8534	0.6416	0.7524	0.8004	0.7543	0.6075	0.6432	0.6229	0.6466
0.9499	1.0431	1.1093	1.087	0.8717	0.9214	0.9806	0.8942	0.6765	0.7982	0.8461	0.8031	0.6385	0.6896	0.6782	0.6946
0.988	1.0821	1.156	1.1406	0.9126	0.9693	1.0311	0.9332	0.7148	0.8435	0.8941	0.8543	0.6515	0.7397	0.7286	0.7453
1.0237	1.1263	1.2023	1.1869	0.9549	1.0141	1.0791	0.9748	0.7533	0.8908	0.9393	0.9031	0.715	0.7874	0.7766	0.7942
1.0588	1.167	1.2477	1.2317	0.9968	1.0631	1.1256	1.019	0.7894	0.9307	0.9862	0.9511	0.7514	0.8387	0.8276	0.8395
1.095	1.2024	1.29	1.2745	1.0361	1.1019	1.1696	1.0621	0.824	0.9717	1.0319	0.9977	0.7968	0.8855	0.8755	0.8847
1.1262	1.2368	1.3291	1.3149	1.0706	1.1466	1.2103	1.1022	0.8576	1.0146	1.0762	1.0417	0.7823	0.9251	0.9215	0.9297
1.1499	1.2734	1.3676	1.3543	1.1079	1.1859	1.2506	1.1471	0.8904	1.0537	1.1176	1.0869	0.8233	0.9771	0.9676	0.9736
1.1769	1.3093	1.4046	1.3917	1.1445	1.2209	1.293	1.1856	0.9253	1.0904	1.1585	1.1277	0.8551	1.0233	1.0121	1.0158
1.2036	1.3414	1.4401	1.4246	1.1804	1.2567	1.3326	1.2281	0.9566	1.129	1.1981	1.1682	0.8926	1.0657	1.0533	1.0568
1.2294	1.3758	1.476	1.4569	1.2149	1.2927	1.3713	1.2636	0.9873	1.1685	1.2363	1.2115	0.9203	1.1052	1.096	1.0993
1.2582	1.4083	1.5085	1.4857	1.2491	1.3269	1.408	1.3003	1.0168	1.2045	1.274	1.2493	0.9556	1.1479	1.1368	1.1373
1.2813	1.4414	1.5418	1.5181	1.2844	1.3605	1.4455	1.3292	1.0462	1.2414	1.3119	1.2882	0.9875	1.1806	1.1772	1.1765
1.3072	1.4777	1.575	1.5478	1.3188	1.3948	1.4821	1.3655	1.078	1.2767	1.3485	1.3251	1.0183	1.2267	1.2169	1.2144
1.3325	1.5084	1.6112	1.5821	1.3539	1.4258	1.5187	1.3994	1.1053	1.3075	1.3841	1.3618	1.0488	1.2623	1.2540	1.2523
1.3605	1.5416	1.6402	1.6073	1.3874	1.4575	1.5513	1.4306	1.1367	1.3419	1.4183	1.3977	1.0793	1.2999	1.2907	1.2895
1.3847	1.5708	1.6726	1.6435	1.4192	1.4879	1.5825	1.4643	1.1648	1.3755	1.4512	1.4326	1.1102	1.3341	1.327	1.3268
1.4128	1.5991	1.699	1.6767	1.4528	1.5165	1.6161	1.4914	1.1941	1.4061	1.4834	1.4675	1.1385	1.3664	1.3609	1.3619
1.4400	1.6315	1.7279	1.7072	1.4816	1.5455	1.6482	1.5232	1.2215	1.439	1.5163	1.4998	1.1671	1.4022	1.3946	1.395
1.4634	1.6571	1.7574	1.7389	1.5068	1.5721	1.6792	1.552	1.2483	1.4694	1.5472	1.5328	1.1947	1.4312	1.4274	1.4278
1.4904	1.6826	1.7856	1.7706	1.5416	1.6005	1.7074	1.5802	1.2764	1.4985	1.5767	1.5631	1.2223	1.461	1.4576	1.4587
1.5145	1.7082	1.8086	1.799	1.5629	1.6271	1.7353	1.6055	1.3015	1.5253	1.6054	1.593	1.2477	1.4903	1.4864	1.4904
1.5409	1.7303	1.8383	1.8288	1.5931	1.6536	1.7624	1.6318	1.3256	1.5539	1.6327	1.6225	1.2746	1.5161	1.5174	1.5197
1.5639	1.7555	1.861	1.8559	1.6189	1.6788	1.7898	1.6619	1.3527	1.5782	1.6615	1.6516	1.3009	1.5419	1.5442	1.5491
1.5847	1.7827	1.8857	1.8795	1.6448	1.7023	1.8179	1.6824	1.3772	1.6041	1.6864	1.6776	1.3231	1.566	1.5693	1.5752
1.6075	1.808	1.9083	1.9022	1.6672	1.7258	1.8393	1.7062	1.3986	1.627	1.7113	1.70281	1.3487	1.5887	1.594	1.6012
1.63	1.8316	1.9306	1.9235	1.6924	1.7498	1.8634	1.7323	1.4246	1.6532	1.7341	1.7272	1.374	1.6105	1.618	1.6255
1.6542	1.8508	1.951	1.9453	1.7098	1.7707	1.8844	1.7556	1.4478	1.6732	1.7581	1.751	1.3965	1.6319	1.6426	1.6489

0.065182	0.076619	0.081546	0.074251	0.0808	0.081557	0.087656	0.08283	0.072356	0.084034	0.087518	0.091593	0.073623	0.092245	0.094047	0.092151
0.070900281		0.077898419		0.081178237		0.085242657		0.078194783		0.089555705		0.082933719		0.093098982	
0.0744				0.0832				0.0839				0.0880			
37.13867	26.10825	21.35712	28.39173	22.0766	21.34611	15.46439	20.11883	30.21997	18.95725	15.59692	11.66725	28.99792	11.0388	9.301006	11.12886
28.2489				19.7515				19.1103				15.1166			
6.6113				2.9700				7.9831				9.2923			

APPENDIX D

Certificates of Analysis/ Service Reports

Certificate of Analysis for TPC/TFC Assay

COLLEGE OF AGRICULTURE AND FOOD SCIENCE
UNIVERSITY OF THE PHILIPPINES LOS BAÑOS
Institute of Plant Breeding, UP Los Baños
College, Laguna, 4031
(049) 547 0654 | ipb.uplb@up.edu.ph
+63 956 6871 056 | +63 930 0798 180

INSTITUTE OF PLANT BREEDING

Analytical Services Laboratory
Summary of Results

Request Number: 24-IPB-ASL-137-1205
Sample Type: Extracts
Number of samples: 4
Analysis requested: Total phenols, Total flavonoids

Requested by: Kris Allen Pangilinan
pangilinan2200625@ceu.edu.ph

Table 1. Total phenols and total flavonoids content of *V. globosa* extracts.

Sample	Total phenols, mg GAE/mL	Total flavonoids, mg CE/mL
V. globosa ethyl acetate fraction	24.78 ± 1.39	5.14 ± 0.39
V. globosa hexane fraction	1.79 ± 0.04	0.59 ± 0.03
V. globosa aqueous fraction	12.40 ± 0.80	0.68 ± 0.06
V. globosa crude extract	13.92 ± 0.97	2.20 ± 0.04

Prepared by:

Earlene E. Lagasca
PRC Number: 0015439
Analyst, CAFS-IPB ASL

Certificate of Analysis for DPPH Radical Scavenging Assay

NATIONAL INSTITUTE OF MOLECULAR BIOLOGY
AND BIOTECHNOLOGY (BIOTECH)
UNIVERSITY OF THE PHILIPPINES LOS BAÑOS

UPLB S&T Park, UP Los Baños, College, Laguna 4031 Philippines
+63 947 5859 416 | biotech.uplb@up.edu.ph

CERTIFICATE OF ANALYSIS
Food Laboratory I
Email: foodlab1.uplbbiotech@gmail.com

Client Name: Kris Allen E. Pangilinan
School/Institution: Centro Escolar University Manila
Service Required: Determination of DPPH radical scavenging activity
Type of Sample/s: Ethyl acetate fraction of *Voacanga globosa*
Number of Sample/s: 1
Date of Sample Submission: 11 February 2025
Date Analyzed: 17 February 2025
Date Reported: 17 February 2025

RESULTS:

Table 1. DPPH radical scavenging activity of ethyl acetate fraction of *Voacanga globosa* and ascorbic acid.

Sample	% Inhibition at varying concentrations (µg/ 100 µL)					
	10	15	20	25	30	Stock Solution
Ethyl acetate fraction of *Voacanga globosa*	2.40	4.40	5.20	6.80	8.24	22.40
	2.64	4.64	5.36	7.04	8.64	22.64
	2.80	4.88	5.36	7.44	8.72	22.64
Average	**2.61**	**4.64**	**5.31**	**7.09**	**8.53**	**22.56**
Ascorbic acid	19.12	31.32	41.29	48.71	63.79	-
	20.31	30.92	41.45	48.95	62.35	-
Average	**19.72**	**31.12**	**41.37**	**48.83**	**63.07**	-

% Inhibition is the DPPH radical scavenging activity indicated by the decrease in absorbance relative to the control (without inhibitor).
Concentrations are expressed as per 100 µL assay. The concentrations are also equal to 0.10, 0.15, 0.20, 0.25, and 0.30 µg/µL.
Stock solution refers to the concentrated form which has a concentration of 98.40 µg/100µL or 0.9840 µg/µL.

Disclaimers:

1. The sample name or code is provided by the client. This report does not confirm or verify how the sample was extracted or prepared prior to submission.
2. Food Laboratory 1 does not guarantee specific results from analyses. Results are based on the specific sample provided and are subject to variation based on sample type, condition, and preparation.
3. The laboratory assumes no liability for decisions or outcomes resulting from the use of this report, as its interpretation and application are the client's responsibility. Misuse or misinterpretation is excluded from the laboratory's responsibility.
4. This report shall not be reproduced in part or in full without written permission from Food Laboratory 1.
5. The use of BIOTECH and UPLB seal and name is strictly prohibited unless authorized by the UP proper authorities.

..... Nothing follows .....

Technical Analyst:	Certified by:	Noted By:
JOHN KENNETH A. VILLASIN	MARIA KATRINA N. ALAON, M.Sc.	FILES MARCIANA Z. TAMBALO, M.Sc.
Junior Research Associate	University Researcher II	Director

Page 1 of 1

#202502-TK9119

Certificate of Analysis for CUPRAC Antioxidant Capacity Assay

NATIONAL INSTITUTE OF MOLECULAR BIOLOGY
AND BIOTECHNOLOGY (BIOTECH)
UNIVERSITY OF THE PHILIPPINES LOS BAÑOS

UPLB S&T Park, UP Los Baños, College, Laguna 4031 Philippines
+63 947 5859 416 | biotech.uplb@up.edu.ph

CERTIFICATE OF ANALYSIS
Food Laboratory I
Email: foodlab1.uplbbiotech@gmail.com

Client Name: Kris Allen Pangilinan
School/Institution: Centro Escolar University
Service Required: Determination of antioxidant property via cupric reducing antioxidant capacity (CUPRAC) method
Type of Sample/s: Ethyl acetate fraction of *Voacanga globosa*
Number of Sample/s: 1
Date of Sample Submission: 11 February 2025
Date Analyzed: 12 February 2025
Date Reported: 13 February 2025

RESULTS:

Table 1. Cupric reducing antioxidant capacity of ethyl acetate fraction of *Voacanga globosa*.

Sample	% Inhibition at varying concentration** (µg / 500 µL)				
	10	15	20	25	30
Ethyl acetate fraction of *Voacanga globosa*	0.067	0.122	0.144	0.200	0.248
	0.070	0.128	0.145	0.201	0.246
	0.071	0.118	0.143	0.201	0.247
Average	**0.069**	**0.123**	**0.144**	**0.201**	**0.247**
Ascorbic acid	0.253	0.369	0.486	0.608	0.670
	0.257	0.374	0.490	0.610	0.671
	0.254	0.369	0.486	0.603	0.672
Average	**0.255**	**0.371**	**0.487**	**0.607**	**0.671**

*Absorbance is directly related to reducing power; higher absorbance, more potent.
**Concentrations are expressed as per 500 µL assay. The concentrations are also equal to 0.02, 0.03, 0.04, 0.05, and 0.06 µg/µL.

Disclaimers:
1. The sample name or code is provided by the client. This report does not confirm or verify how the sample was extracted or prepared prior to submission.
2. Food Laboratory 1 does not guarantee specific results from analyses. Results are based on the specific sample provided and are subject to variation based on sample type, condition, and preparation.
3. The laboratory assumes no liability for decisions or outcomes resulting from the use of this report, as its interpretation and application are the client's responsibility. Misuse or misinterpretation is excluded from the laboratory's responsibility.
4. This report shall not be reproduced in part or in full without written permission from Food Laboratory 1.
5. The use of BIOTECH and UPLB seal and name is strictly prohibited unless authorized by the UP proper authorities.

..... Nothing follows .....

Technical Analyst:	Certified by:	Noted By:
JOHN KENNETH A. VILLASIN	MARIA KATRINA N. ALAON, M.Sc.	FIDES MARCIANA Z. TAMBALO, M.Sc.
Junior Research Associate	University Researcher II	Director

Page 1 of 1

202502 - FEC/119

Acetylcholinesterase Inhibition Assay Screening Report

Terrestrial Natural Products Laboratory
Institute of Chemistry
College of Science
University of the Philippines
Diliman, Quezon City 1101

Rm. 301-302, Institute of Chemistry, Research Building, National Science Complex
UP Trunkline 8981-85-00 Loc. 3655 Email address: tnpl.upd@gmail.com, tnpl.upd@up.edu.ph

SERVICE REPORT

TNPL Reference No:	UPD-ACHE-250311
Requesting Agency:	Kris Allen E. Pangilinan
Affiliation:	Centro Escolar University Manila
No. of Samples:	1 sample (10 concentrations)
Service Requested:	**Acetylcholinesterase Inhibition Assay**
Date Assayed:	March 11, 2025
Date results were released:	March 14, 2025

BACKGROUND

The assay is based on the action of cholinesterase inhibitor(s) on cholinesterase and the substrate. The enzyme breaks acetylthiocholine iodide into acetate and thiocholine. Liberated thiocholine reacts with the added DTNB dye to produce TNB, a yellow compound that is monitored spectrophotometrically. The rate at which the yellow color changes is associated with enzyme activity. In the presence of an inhibitor, the structure of the substrate is retained and the inhibitor binds to the enzyme, slowing the rate of reaction.

METHODOLOGY

A sample stock solution of 20,000 ppm was prepared and working solutions were prepared via serial dilution. Samples were homogenized using a vortex mixer, sonicated, and centrifuged. In a 96-well plate, the reaction mixture consists of 180 µL of 100 mM phosphate buffer at pH 8.0, 10 µL of 1.0 U/mL *Electrophorus electricus* acetylcholinesterase (AChE) enzyme solution, 80 µL of buffered Ellman's reagent (10.0 mM DTNB and 17.9 mM $NaHCO_3$ at pH 7.0), and 15 µL of each working solution sample/100 ppm positive control/negative control (methanol). Each test sample solution has two trials and two replicates (n=2, t=2). The sample, positive control, and negative control has a solvent well concentration of 5% methanol. After incubation for 15 minutes at 25°C, 15 uL of 2.87 mM acetylthiocholine iodide (AChI) was added and the absorbance was monitored at 420 nm using Multiskan (Thermo Scientific) for 31 readings with 25-s intervals.

The inhibitory activities of the samples and the positive control (galantamine) were determined based on the average slope of each replicate using equation 1.

$$\%\ Inhibitory\ activity = \frac{Slope_{uninhibited} - Slope_{inhibited}}{Slope_{uninhibited}}\ x\ 100 \qquad (1)$$

where $slope_{uninhibited}$ is the slope of the line from the absorbance vs time plot of the negative control group and the $slope_{inhibited}$ is the slope of the line from the absorbance vs time plot of the samples or positive control.

A sample is considered active if the % inhibition is greater than or equal to 50% and if there is a significant mean difference with negative control at $p < 0.05$. Kolmogorov-Smirnov Test, Levene's test, Brown-Forsythe Test, Welch Test, and One-Way ANOVA using SPSS 25.0 were employed to statistically analyze the data. Active samples are highlighted in Table 1.

REFERENCE

Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*, 7:88-95.

RESULTS

Table 1. Acetylcholinesterase inhibitory activity at several concentrations. Highlighted samples are active.

Sample No	Sample Code	% inhibition	SD (± %)
1	1000 ppm	68.39263991*	9.478078394
2	500 ppm	36.03941407	0.671125185
3	450 ppm	42.99325392	5.443672871
4	400 ppm	28.24894501	4.772284699
5	350 ppm	19.11037882	7.747388267
6	300 ppm	33.50862185	0.376758696
7	250 ppm	46.41137632	0.466180879
8	200 ppm	31.99915702	6.429457364
9	150 ppm	19.75148239	2.771678136
10	100 ppm	15.11664694	6.932066597
Galantamine 100 ppm		99.31546227*	0.11093576

* mean difference is significant at p>0.05

Of the 10 analyzed concentrations of the sample, a significant inhibitory activity against acetylcholinesterase was observed at 1000 ppm.

Performed by

Camille Ann F Sapico
Researcher-in-charge
PRC License No. 0012902

Noted by

Evangeline C Amor, PhD
Lab Manager
PRC License No. 0007099

CERTIFICATION OF STATISTICAL TREATMENT

This is to certify that the statistical treatment for the study titled ***IN- VITRO ANALYSIS OF THE ANTIOXIDANT AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF THE FLAVONOID-RICH FRACTION OF BAYAG-USA (VOACANGA GLOBOSA) LEAF EXTRACT*** upon the request of **KRIS ALLEN PANGILINAN**, was performed with utmost diligence and adherence to established statistical standards.

File Control Number: **STAT-12030525**

This certification is provided to ensure integrity and validity of the statistical treatment conducted by **RICARDO JR. N. ARELLANO, RPh**. For any further inquiries or clarifications, please do not hesitate to contact at **rnarellano@ceu.edu.ph**.

Ricardo Jr. N. Arellano
Faculty member,
Centro Escolar University
Mendiola, Manila

APPENDIX E

Documentation from the entire Plant preparation process from Collection until Solvent Partitioning

Image 1: Collection. Collecting plant sample leaves was done in Alaminos, Laguna.

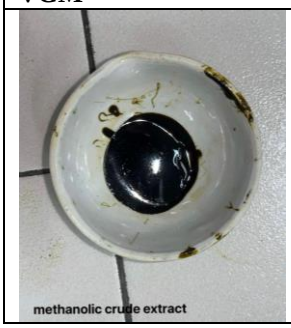
Image 2: Drying. Plant sample leaves of bayag-usa were shade-dried at room temperature for a five-day period.

Image 3: Blending. Dried plant sample leaves were crushed and grinded into a fine powder using a blender.

Image 4: Maceration. The powdered leaves were soaked in an adequate amount of 80% methyl alcohol, ensuring complete submersion. Plant sample was macerated for a period of 24 to 48 hours.

Image 5: Solvent Partitioning. Using solvent fractionation, the methanolic extract yields aqueous, ethyl acetate, and hexane fractions.

Documentation of Dried Samples

VGM	VGH	VGE	VGA
			

APPENDIX F

Documentation for DPPH Radical Scavenging Assay

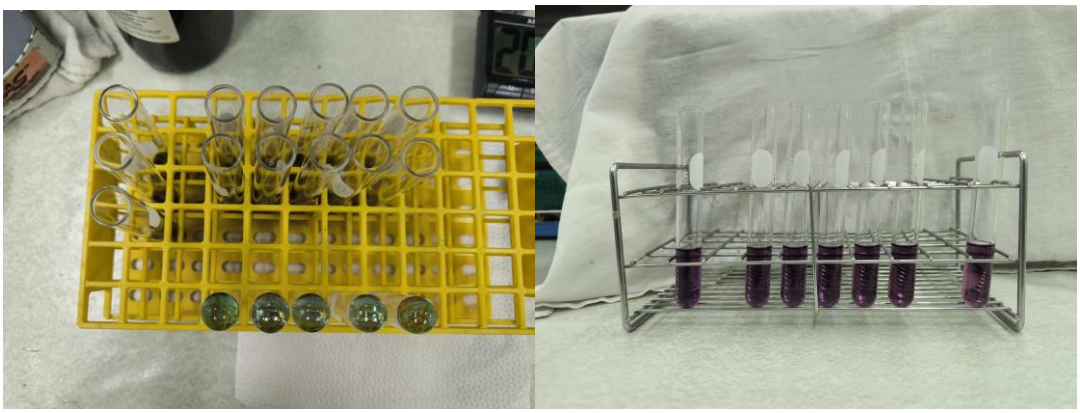


Image 6: The sample was reconstituted in methanol, stirred, and diluted in various concentrations.

Image 7: Different concentrations of the sample were mixed with 5 mL of 0.1 nM DPPH solution.

Image 8: The absorbance of the reaction mixture was measured at 517 nm using a double-beam UV-Vis spectrophotometer.

APPENDIX G

Documentation for CUPRAC Antioxidant Capacity Assay

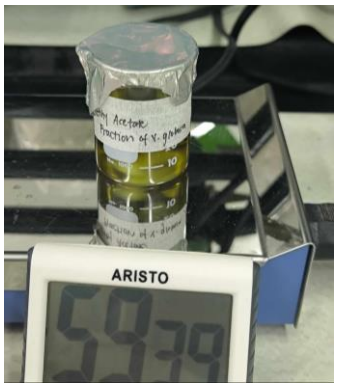


Image 9: The sample was reconstituted in methanol, stirred, and diluted in various concentrations.

Image 10: Addition of $CuCl_2$, ammonium acetate, neocuproine, and sample in a test tube.

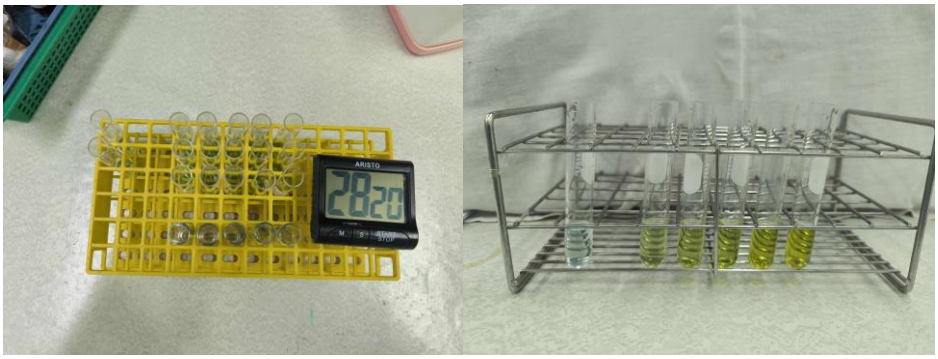


Image 11: The sample was incubated for 30 minutes to allow formation of colored complexes.

Image 12: The activity of the sample was quantified by measuring its absorbance at 450 nm using a spectrophotometer.

APPENDIX H

Documentation for Acetylcholinesterase Inhibition Assay

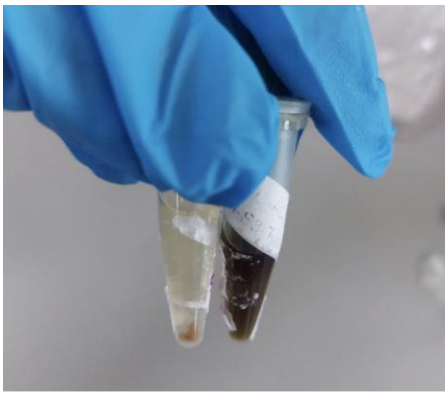


Image 13: Preparation of samples. Sample on the left was only partially soluble to solvent while the right was completely soluble.

Image 14: Dispensation of 180uL PBS buffer pH 8.0 into the 96-well plate using a multichannel pipettor.

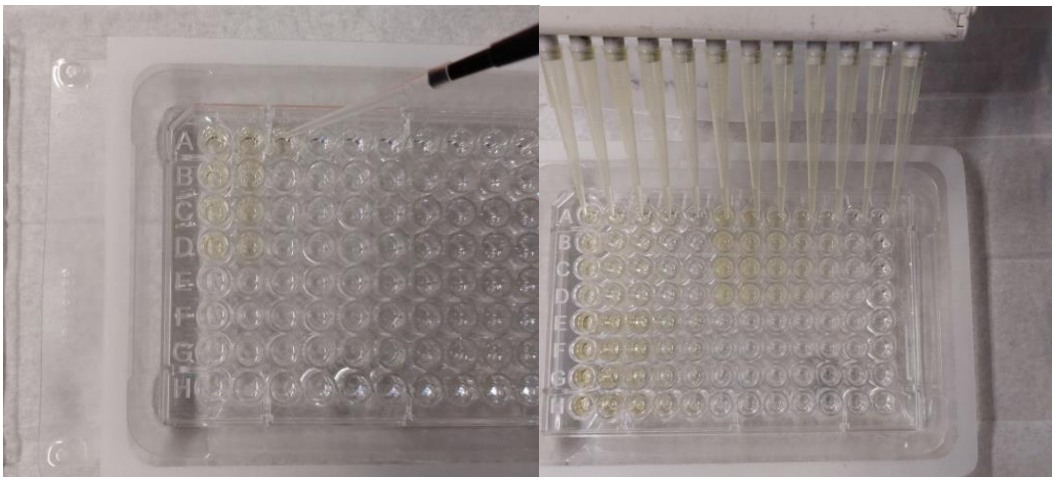


Image 15: (Left) Dispensation of 15uL samples 0 into the 96-well plate using a single channel pipettor. (Right) Dispensation of 10uL acetylcholinesterase enzyme (AChE) into the 96-well plate using a multichannel pipettor.

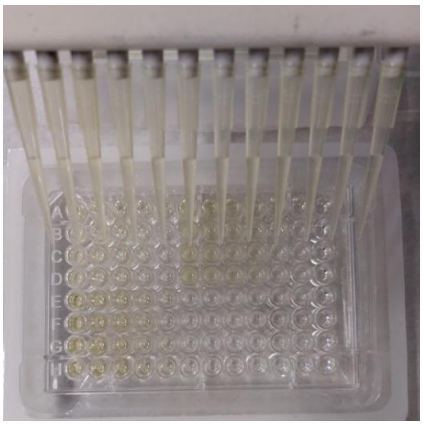


Image 16: Dispensation of 80uL Ellman's reagent into the 96-well plate using a multichannel pipettor.

Image 17: (Left) Incubation of well mixture for 15 minutes using Multiskan (Thermo Scientific). (Right) Dispensation of 15uL substrate (acetylcholine iodide) into the 96-well plate after the incubation.

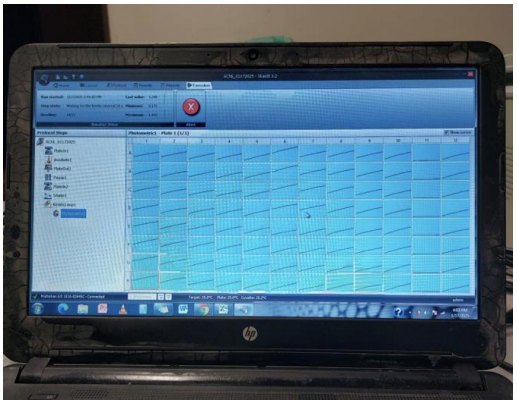


Image 18: Reading of absorbance at 420 nm using Multiskan (Thermo Scientific).

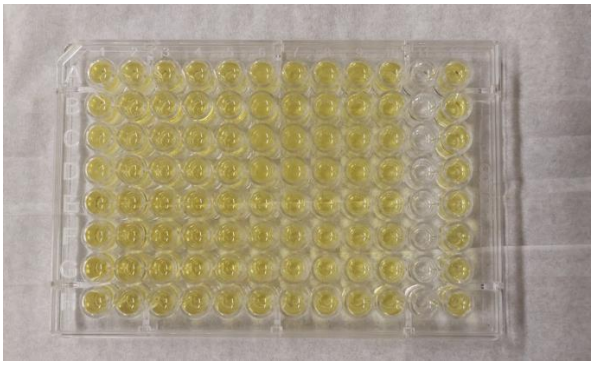


Image 19: Well plate after the assay. Positive control was dispensed in row 11.

APPENDIX H

Research Budget

Research Budget			
Specific Item	Quantity	Price per Unit	Subtotal
SOLVENTS			
99% Methanol	1 (2.5L)	Php 756	Php 756
n-hexane	1 (4L)	Php 2,100	Php 2,100
Ethyl Acetate	1	Php 2,400	Php 2,400
ASSAYS			
Total Flavonoid Content (TFC) Assay		Php 1,250.00/sample	Php 5,000
Total Phenol Content (TPC) Assay		Php 1,250.00/sample	Php 5,000
DPPH Radical Scavenging Assay		Php 1,250.00/sample	Php 1,250
Cupric Reducing Antioxidant Capacity (CUPRAC) Assay		Php 2,500/sample	Php 2,500
Acetylcholinesterase (AChE) Inhibition Assay + Documentation		Php 12,4500.00/sample	Php12,450
MISCELLANEOUS FEES			
Laboratory Materials			Php 2,000
Transportation and other expenses			Php 3,000
TOTAL			Php 36,456

APPENDIX I

Gantt Chart

TASK	AUGUST				SEPTEMBER				OCTOBER				NOVEMBER			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Research topic brainstorming and Reviewing of Literature																
Topic Proposal																
Collection and Authentication of Plant Sample																
Preparation of Crude Extract																
Phytochemical Screening																
Research Proposal Presentation																
Preparation of Crude Extract for Biological Assays																
Solvent Partitioning																
Phytochemical Screening of Fractions																
Finals Presentation																
PAPERWORKS																
Chapter 1																
Chapter 2																
Chapter 3																
Chapter 4																
Chapter 5																

TASK	JANUARY				FEBRUARY				MARCH				APRIL			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
TFC/TPC Assay																
DPPH Assay																
CUPRAC Assay																
AChE Assay																
Abstract Drafting																
Powerpoint Presentation Drafting																
Abstract Journal Drafting																
PAPERWORKS																
Chapter 1																
Chapter 2																
Chapter 3																
Chapter 4																
Chapter 5																

APPENDIX J

Plagiarism Scan